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Female	75.8	77.1	78.0	78.6	79.9	81.9	83.9	85.4	86.8	86.8	84.4

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*Blood Pressure, Report of Joint Committee on Mortality of The Association of Life Insurance Medical Directors and The Actuarial Society of America. New York, 1925. pp. 10, 52.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

MARCH, 1948

ORIGINAL ARTICLES

STREPTOMYCIN IN THE TREATMENT OF TULAREMIA

By Robert C. Berson, M.D.*

* Now in private practice in Rye, New York.

AND

Aubrey B. Harwell, M.D.†

NASHVILLE, TENNESSEE

(From the Medical Service, Thayer Veterans Administration Hospital)

The purpose of this report is to summarize the clinical data on 56 cases of tularemia which have been treated with streptomycin in 22 Veterans Administration Hospitals.

Since its first description in 1911,[20] tularemia has been the subject of intense and rewarding study by a large number of workers. Its etiology,[11,29] pathology,[19,28] epidemiology,[3,5,13,30] and clinical course[7,23] have been clearly described and the American literature has become so rich on all phases of the subject up to the introduction of streptomycin that we feel it superfluous to add to this material. The reader is referred to one of the several excellent reviews of the literature.[7,11,21,23]

Shortly after Schatz, Bugie, and Waksman[24,25] discovered and described streptomycin and suggested its use in the treatment of diseases caused by a variety of Gram-negative pathogens, Heilman[12] reported its effectiveness against *P. tularense* both *in vitro* and in rats. From March to July, 1946, the Committee on Chemotherapeutics and other Agents of the National Research Council distributed the antibiotic for clinical trial in a number of different hospitals and, in consequence, published reports of its use in human infection with *P. tularense* appeared simultaneously from several different workers. Foshay[10] reported in detail his observations on 7 cases and later[8] extended them to 9 cases. Keefer[17] includes 67 cases in his official report on streptomycin, but goes into no detail concerning them. Nichols and Herrell[22] list 15 cases in their general report, Howe *et al.*[15] discuss 7 cases, Abel[1] had 3 cases and Cohen and Lasser[4] reported 1 case.

Commencing in March, 1946, and for the remainder of the calendar year, the Streptomycin Committee of the Central Office, Veterans Administration, in Washington, D. C., shipped streptomycin by Air Express to its hospitals upon telegraphic notification of appropriate diagnoses. Among the cases thus treated were 56 patients with tularemia. At the request of the Streptomycin Committee and through the extraordinary courtesy and coöperation of the various hospitals and the individual doctors who treated the patients, we have been able to review in detail their complete clinical records for the purpose of this publication.

The clinical classification of human infection with *P. tularense* into ulceroglandular, oculoglandular, glandular, and ty-

† Instructor in Clinical Medicine at Vanderbilt University School of Medicine.

phoidal types[7] has served useful purposes in the study of the clinical course,[2,7,16,18,26,27] mode of infection,[3,13] the rôle of insect vectors,[5,13] and pathology.[19] Blackford and Cassey,[2] and Pullen and Stuart[23] and others[7,16,18,26,28] have emphasized the frequency and the importance of pneumonic involvement in all varieties of this infection. In our group of patients the occurrence of pleuropulmonary tularemia, with or without obvious involvement elsewhere, added so much to the gravity and urgency of the clinical problem that it seems desirable to divide the cases into a group of 15 who had notable pneumonic involvement, and a group of 41 without pulmonary infection.

TABLE 1.—SUMMARY OF CLINICAL DATA IN 41 CASES OF ULCEROGLANDULAR TULAREMIA TREATED WITH STREPTOMYCIN

Case No.	Day of illness drug started	Total dose (gm.)	No. of treatment days	Average maximum temperature before treatment	After beginning treatment			Condition of lymph nodes at discharge
					Days to clinical improvement	Days of fever	No. of days to discharge	
1	3	6.0	5	100.0	1	4	6	Regressing
2	4	8.4	7	100.0	1	2	17	Normal
3	4	7.0	6	104.0	1	5	8	Normal
4	5	8.0	8	98.8	?	?	15	Normal
5	7	13.2	8	102.0	1	5	17	Normal
6	9	8.0	8	98.4	?	?	27	Normal
7	9	8.0	6	101.0	1	1	14	Normal
8	10	8.8	11	103.0	1	2	13	Normal
9	10	6.0	7	100.4	1	2	8	Regressing
10	12	9.6	8	101.4	1	2	36	Regressing
11	13	4.0	5	102.8	1	2	12	Enlarged
12	13	7.0	4	100.0	4	5	23	Enlarged
13	15	8.0	8	100.8	2	4	53	Draining
14	15	9.1	12	99.4	5	?	14	Regressing
15	15	7.2	6	99.2	?	7	39	Draining
16	16	8.0	6	98.8	?	?	9	Regressing
17	16	9.6	8	98.8	4	4	25	Regressing
18	17	7.0	7	99.2	3?	3	11	Regressing
19	17	8.0	6	?	?	?	15	
20	18	11.5	8	102.1	1	4	18	
21	20	8.0	6	99.5	4	4	14	Regressing
22	21	8.0	8	99.0	?	4	12	Regressing
23	21	12.0	8	100.4	3	4	39	Draining
24	22	4.0	5	99.0	?	?	7	Normal
25	22	11.6	10	101.4	3	8	11	Regressing
26	23	9.0	9	99.8	3	3	35	Regressing
27	25	8.4	7	102.0	2	5	13	Regressing
28	25	20.0	17	99.8	?	6	46	Draining
29	26	10.8	9	99.8	1	2	23	Unchanged
30	29	7.2	6	98.0	?	?	25	Regressing
31	29	8.4	7	99.9	?	?	7	Regressing
32	29	7.0	6	102.2	?	10	26	Regressing
33	30	6.0	6	99.4	?	1	20	Unchanged
34	30	9.6	8	98.4	?	?	9	Regressing
35	32	8.4		100.5	?	?	89	Regressing
36	32	1.92	6	101.6	5	5	49	Draining
37	42	9.6	8	99.2	11	15	51	Draining
38	42	17.6	13	99.0	2	2	41	Draining
39	48	9.6	8	100.0	2	2	8	
40	..	18.6	13	98.4	3	3	71	Regressing
41	90	7.0	7					

Proof of Diagnosis. Absolute proof of etiology in this infection depends upon the isolation of **P. tularense** from the tissues of the patient, or the presence of specific agglutinins, with a rising titer, in the patient's serum. Because of the danger of laboratory infection, few hospitals made serious efforts to recover the organism by culture, and in only 2 cases was the pathogen demonstrated by animal inoculation. In the remainder, the diagnosis in the ulceroglandular cases was suggested by the clinical features of the infection (exposure to possible vectors, primary ulcer, regional lymphadenopathy, and others) and in every case it was confirmed by the presence of specific agglutinins, with rising titer, in the patient's serum. Proof of pneumonic involvement depended upon positive physical findings and serial roentgenograms associated with the presence of specific agglutinins.

Ulceroglandular Tularemia. Forty-one patients with ulceroglandular tularemia were treated with streptomycin. The summary of the clinical data is contained in Table 1.

From the clinical data at our disposal, it is obvious that the criteria for discharge from the several hospitals varied with the individual hospital and with the individual patient. Furthermore, from the clinical records it is impossible to determine the duration of follow-up studies. In general it seems clear that the patients were afebrile for an appreciable period of time, were ambulatory, and were gaining weight, before they were discharged.

TABLE 2.—TIME RELATIONSHIP OF STREPTOMYCIN TREATMENT TO SUBSEQUENT COURSE IN ULCEROGLANDULAR TULAREMIA

Days of illness preceding treatment	No. of patients	After beginning treatment		
		Days to clinical improvement	Days of fever	No. of days to discharge
3-12	10	1.00	3.00	20.1
13-24	16	3.00	4.35	21.06
24+	15	3.83	5.32	30.7

In Table 2 the patients are divided into groups on the basis of the duration of illness before treatment was instituted.

In 10 patients, treatment was begun between the 3rd and 12th day of illness. In several of these cases treatment was instituted before serum agglutinins were demonstrated, but in each instance the history, clinical course, and the physical findings were sufficiently suggestive to warrant treatment. In every case the agglutinins appeared subsequently. Each of these 10 patients showed evidence of clinical improvement within 24 hours. They became afebrile within an average of 3 days, and were discharged from the hospital in an average of 20.1 days after the treatment was instituted. In 7 patients, lymphadenopathy had completely disappeared, while in 3 patients there was notable regression of the lymphadenopathy by the time of discharge.

In 16 patients, treatment was instituted between the 13th and 24th day of illness. The average number of days which elapsed before notable clinical improvement was 3. The patients became afebrile, on an average of 4.3 days, and were discharged from the hospital on an average of 21.1 days after treatment was instituted. In 1 patient the lymphadenopathy had completely disappeared at the time of discharge, and in 8 patients there was notable regression. In 2 patients there had been definite enlargement of the nodes, and in 2 patients with discharging buboes drainage was still present at the time of dismissal from the hospital.

In 13 patients, treatment was begun after the 24th day of their illness. In several of these cases the infection was a low-grade chronic process associated with discharging buboes. Clinical improvement in this group was not as prompt or as impressive as when the infection was treated early. For the 7 patients in whom the time could be determined, the average number of days from the institution of treatment to clinical improvement was 3.8; of the 13 patients 10 exhibited fever. The average number of days required for the disappearance of fever was 5.3. The patients in this group were discharged from the hospital on an average of 30.7 days after treatment was begun. In 7 cases there was notable regression, and in 2 patients there was no change in the lymphadenopathy. Four patients had discharging buboes at the time of their dismissal from the hospital.

Pleuropulmonary Tularemia. Fifteen of the group of 56 patients had frank pleuropulmonary involvement. All were treated with streptomycin. Data regarding these cases are summarized in Table 3.

Table 4 indicates the duration of illness before treatment was instituted in patients with pleuropulmonary involvement, the number of days before improvement was noted, and the duration of fever and of hospitalization after the institution of treatment.

In 4 patients treatment was begun

between the 1st and the 12th day of illness. The average number of days until notable clinical improvement occurred was 1.7. The patients became afebrile, on an average, in 3.7 days and were discharged from the hospital, on an average, in 24 days after the beginning of treatment.

sions as well. The remaining 10 were examples of tularemic pulmonary infection in the absence of ulceroglandular lesions and, therefore, constituted difficult diagnostic problems. The importance of correct etiologic diagnosis in such cases is emphasized by the fact that no deaths occurred in our

TABLE 3.—SUMMARY OF CLINICAL DATA IN 15 CASES OF PLEUROPULMONARY TULAREMIA TREATED WITH STREPTOMYCIN

Case No.	Day of illness drug started	Total dose (gm.)	No. of treatment days	Average maximum temperature before treatment	After beginning treatment: Days to clinical improvement	After beginning treatment: Days of fever	After beginning treatment: No. of days to discharge	Remarks
42	9	5.1	8	104.3	2	6	16	
43	11	6.7	8	103.4	1	2	24	
44	11	8.4	5	100.5	3	3	33	Skin rash attributed to drug
45	12	14.0	7	103.0	1	4	23	
46	14	14.4	7	103.0	1	3	31	
47	14	10.8	9	103.0	1	5	14	
48	15	8.0	5	103.0	1	11	45	
49	17	7.2	6	103.4	1	2	31	
50		6.0	15	103.6	1	15	48	Sixth day of treatment pleural effusion; 12th day thrombophlebitis.
51	23	21.6	9	103.5	1	7	34	Developed pleural effusion. At discharge Roentgen ray interpretation of residual atelectasis. Condition good at discharge.
52	24	21.6	18	102.6	3	4	20	
53	25	7.0	7	102.0	3	14	26	Antiserum prior to streptomycin; 8 days later serum sickness. On streptomycin developed a back abscess requiring drainage.
54	28	6.4	8	101.4	1	1	67	
55	34	13.0	13	99.2	?	4	20	
56	71	7.0	7	100.5	1	3	9	

TABLE 4.—TIME RELATIONSHIP OF STREPTOMYCIN TREATMENT TO SUBSEQUENT COURSE IN PLEUROPULMONARY TULAREMIA

Days of illness preceding treatment	No. of patients	After beginning treatment: Days to clinical improvement	After beginning treatment: Days of fever	After beginning treatment: No. of days to discharge
0–12	4	1.7	3.7	24.0
12–24	7	1.3	6.7	31.8
24–71	4	1.7	5.5	30.5

In 7 patients treatment was begun between the 12th and the 24th day of illness. The average number of days until notable clinical improvement took place was 1.3. They became afebrile in 6.7 days, and were discharged from the hospital in 31.8 days after the beginning of treatment.

In 4 patients treatment was begun after the 24th day of illness. The average number of days until notable clinical improvement occurred was 1.7. They became afebrile in 5.5 days, and were discharged from the hospital in 30.5 days after treatment was begun.

Five of the patients with pleuropulmonary involvement had ulceroglandular le-

group of 15 cases exhibiting this manifestation of the disease.

Mortality. Mortality figures in a series as small as ours are not statistically valid,[7] nevertheless it is certainly noteworthy that in a group of 56 patients with tularemia there was only 1 death, and in this case necropsy indicated that tularemia was not primarily responsible. An abstract of this case follows:

Case Reports. CASE NO. 41. A 51-year old trapper was admitted to the hospital 6 weeks after developing an ulcer on the left index finger followed by left axillary adenopathy, which had required drainage. On admission there was pain in the right upper

quadrant, an enlarged liver, and jaundice. Tularemia agglutinations were found to be positive in a dilution of 1:320 rising to 1:640. He was therefore started on 1 gm. of streptomycin daily with prompt remission of the fever. His course was steadily downhill and he died on the 6th day of therapy. At autopsy a carcinoma of the pancreas was found with metastases to the liver, spleen, and peritoneum.

Over a fourth of our cases with tularemia had pleuropulmonary involvement. After carefully reviewing the literature in 1941, Blackford and Cassey[2] concluded that the mortality from the pleuropulmonary form of tularemia is approximately 30%. Every one of our 15 cases with this form of the disease recovered.

Suppurative Lymphadenitis. An analysis of the records of the 56 patients reveals that in 11 cases suppurative lymphadenitis occurred. In 1 case, spontaneous drainage occurred before treatment was instituted; in 10 it became manifest during or after the completion of streptomycin treatment. In each instance, the dosage of streptomycin and the duration of its administration were adequate to produce marked improvement in all of the other manifestations of the disease.

Two of these patients with suppurative lymphadenitis were of special interest because they received 1 gm. of streptomycin daily over a period of several days, but later developed exacerbations of their disease requiring additional therapy. Brief summaries are presented:

Case No. 40. A 25 year old man was admitted to the hospital with a firm, tender mass in the left epitrochlear area and another in the left axilla. Streptomycin therapy was begun on the 19th day of illness and continued at the rate of 1 gm. daily for 9 days. The epitrochlear node progressed to suppuration and was aspirated while the axillary node regressed very slowly. Two days after streptomycin was discontinued, there was enlargement and increased tenderness of both involved nodes. Ten days after the first course was discontinued, a second course was begun in the same dosage and continued for 9 days. The induration surrounding the nodes again regressed but the nodes themselves progressed to suppuration and both required drainage 6 days after the second course of the drug was completed.

Case No. 38. A 44 year old man was admitted with left inguinal adenopathy, one node being fluctuant at the time of admission. Streptomycin was begun on the 42nd day of illness at the rate of 1 gm. per day, and there was prompt remission of his local signs and symptoms. Nine days after completion of the first course of therapy tenderness, swelling, and induration again became apparent, with one node becoming fluctuant. On the 18th day after completion of the first course, a second course was begun at the rate of 1 gm. daily for 7 days. The fluctuant nodes progressed to spontaneous rupture, while there was prompt regression of the remainder of the nodes.

Our experience indicates that the bubo is the most refractory of all tularemic lesions to streptomycin treatment. Slow resolution of the primary ulcer and of pneumonic and pleural reactions have been noted and attributed to the granulomatous nature of the infection. However, the actual progression of lesions of the lymph nodes with liquefaction and discharge while the patient is under streptomycin treatment, or after recovery from systemic manifestations of infection and cessation of treatment, is unique. The explanation of this failure probably resides in the granulomatous nature of the tularemic inflammatory process, the inability of the body fluids to deliver the streptomycin in adequate concentration to the interior of the inflamed lymph node, and the necrosis which may have taken place before the institution of specific therapy. Foshay[9] has suggested that a secondary infection of the lymph node is the responsible factor.

Toxic Reactions. Only 3 patients developed skin rashes attributable to streptomycin. Case No. 15 developed urticaria on the 4th day of streptomycin therapy which did not necessitate discontinuing the drug. Case No. 23 developed a slightly raised erythematous rash across the shoulders on the 2nd day of therapy. The drug was continued for an additional 7 days during which time the rash was treated locally and subsided promptly. Case No. 44 developed a pruritic rash on

the trunk and extremities on the 3rd day of streptomycin therapy. Treatment was continued for an additional 2 days and then discontinued because of general improvement. The rash lasted in this patient for a total of 5 days and then cleared without sequelæ.

Immediate Response to Treatment. In those patients whose illness pursued a stormy course there was invariably prompt and dramatic clinical improvement shortly after streptomycin was begun. The temperature usually became normal promptly. Commonly, after the beginning of streptomycin therapy, the induration and tenderness about the involved lymph nodes disappeared, whether the node itself was fluctuant or not. In most instances the patient promptly developed a feeling of well being which was reflected in disappearance of "toxicity," improvement of the appetite, and gain in weight.

Dosage of Streptomycin and Duration of Treatment. The total dosage used in the 56 cases varied from 1.9 gm. to 20 gm., averaging 8.1 gm. There was no difference in the dosage used in the ulceroglandular and the pleuropulmonary cases.

The duration of treatment varied from 4 to 18 days, averaging 9.1 days The pleuropulmonary cases were treated an average of 2 days longer than the ulceroglandular cases.

No correlation between the duration of treatment and the completeness of recovery can be shown in our study, partly because treatment was continued for much longer periods in those cases in which fluctuant lymph nodes were present.

Brief summaries of 2 cases in which streptomycin was administered for short periods of time and the patients suffered relapses are presented:

Case No. 16. A 21 year old trapper experienced the onset of chills, fever, and headache with lymphadenopathy in the right. epitrochlear and axillary groups, and a characteristic skin lesion on the right index finger 6 days after his last known exposure. On the 6th day of his illness he was admitted to a private hospital and started on streptomycin, receiving 0.125 gm. every 3 hours. After he had received 16 doses (2 gm.) he was free of symptoms. He left the hospital and resumed his normal activity. Three days later chills, fever, headache, and malaise recurred. He was admitted to the same hospital and started on the same dosage of streptomycin. After he had received a second course of 16 doses (2 gm.) he again experienced marked symptomatic improvement and was discharged to return to work. Four days later he again experienced chills, fever, headache, and axillary tenderness. At this point he was admitted to a Veterans Administration Hospital. He was given 1.25 gm. of streptomycin every 24 hours for 6 days (7.5 gm.) with complete and permanent recovery.

Case No. 19. A 34 year old trapper experienced the onset of headache, chills, fever, and axillary tenderness 4 days after his last known exposure. He had a questionable skin lesion on the right hand. On the 6th day of his illness he was given streptomycin in a private hospital. He received 0.125 gm. of streptomycin every 3 hours. After he had received 12 doses (1.5 gm.) his symptoms had improved so markedly that he left the hospital and returned to work. Three days later he developed chills, fever, axillary tenderness, and malaise, and was admitted to a Veterans Administration Hospital. He was found to have tender nodes in the right axilla and a white blood count of 11,600. Agglutinins against *P. tularense* were present in a dilution of 1:1280. He was given 1.25 gm. of streptomycin daily for 6 days and promptly recovered.

That the duration of treatment is perhaps as important as the total dose of streptomycin is suggested by comparing the result in Case No. 36 with that in Cases No. 16 and No. 19.

Case No. 36. A 51 year old tailor experienced the onset of fever, chilly sensations, and tender swelling in the left groin 5 days prior to his admission. Examination revealed temperature of 103.8, tender lymphadenopathy in the left groin, a white blood count of 13,300, serum agglutinins against *P. tularense* in dilution of 1:20, with subsequent rise to 1:4000 on the 26th day of his illness. On the 32d day of illness he was started on streptomycin 0.04 gm. every 3 honrs contiuued over a period of 6 days (1.92 gm.). There was marked clinical improvement and he became afebrile on the

5th day of his treatment; lymphadenopathy disappeared and recovery was complete.

Four of our cases of pleuropulmonary tularemia received only 0.4 to 0.8 gm. of streptomycin every 24 hours for periods of from 5 to 7 days. Three of these patients were gravely ill at the time treatment was begun. All 4 experienced prompt improvement and proceeded to complete recovery.

Summary. The records of 56 patients with tularemia treated with streptomycin in Veterans Administration Hospitals have been reviewed. Of this group, 15 cases were examples of pleuropulmonary tularemia. The pleuropulmonary infection occurred as a complication of the ulceroglandular form of the disease in only 5 cases. In the remaining 10, no primary site of infection other than the lung or the pleura could be determined.

The only death which occurred in the series was attributed to carcinoma of the pancreas.

Streptomycin, when administered after the 12th day of illness, appears to have little effect upon the course of suppurative lymphadenopathy. Liquefaction and spontaneous drainage of lymph nodes may occur under such circumstances, even though there is striking improvement in the other manifestations of the disease.

Only 3 patients demonstrated sensitivity to streptomycin. In none of these cases was it necessary to discontinue the drug.

Streptomycin was highly effective in the treatment of every case of tularemia in this series. In the acutely ill patient pursuing a stormy course, its effect was most dramatic.

The dosage and duration of streptomycin administration varied considerably in the cases reviewed.

We wish to express our appreciation for the courtesy and coöperation of the Managers and Medical Staff of the following Veterans Administration Hospitals for permission to include their cases in this review: Whipple, Arizona; Fayetteville, Arkansas; Fargo, North Dakota; Chamblee, Georgia; Hines, Illinois; Marion, Illinois; Fort Benjamin Harrison, Indiana; Topeka, Kansas; Wadsworth, Kansas; Wichita, Kansas; Minneapolis, Minnesota; Jefferson Barracks, Missouri; Dayton, Ohio; Muskogee, Oklahoma; Memphis, Tennessee; Mountain Home, Tennessee; McKinney, Texas; Huntington, West Virginia; Wood, Wisconsin; Cheyenne, Wyoming; and Sheridan, Wyoming. Dr. Arthur M. Walker assisted us in gathering clinical material for study, and Dr. Hugh J. Morgan gave us much valuable advice in constructing this report.

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PROCAINE PENICILLIN G IN OIL

I. Plasma Concentrations; Preliminary Observations on its use in Pneumonia

By William P. Boger, M.D.
ASSISTANT VISITING WARD PHYSICIAN

Jacob E. Oritt, M.D.
ASSISTANT RESIDENT IN MEDICINE

Harold L. Israel, M.D.
ASSISTANT VISITING WARD PHYSICIAN
AND

Harrison F. Flippin, M.D.
VISITING WARD PHYSICIAN

With the Technical Assistance of June E. Heckman
PHILADELPHIA, PENNSYLVANIA

(From the Committee for the Study of Infectious Diseases, the Philadelphia General Hospital)

Prolongation of the effectiveness of a given dose of penicillin would be most desirable. Attempts to prolong the action of the drug have endeavored either to hinder the drug from reaching the circulation by slowing the absorption from the site of its injection, or by retarding the rate of its elimination by the renal tubules.

The use of penicillin suspended in peanut oil and beeswax has been reasonably successful in prolonging therapeutically effective concentrations following single injections of the drug. A quantity of water-soluble salt of penicillin suspended in oil and wax constitutes a reservoir from which penicillin is slowly given up to the body fluids. The beeswax is responsible on the one hand for the prolongation of blood concentrations of penicillin following its injection, and, on the other, the cause of most of the disadvantages attendant upon its use. Beeswax gives rise to allergic manifestations, sterile abscesses, and pain following its injection, and, although severe reactions are few, the occurrence of pain and induration at the site of injection is frequent enough to prejudice many physicians against its use.

During the early development of penicillin, little interest was manifested in many of the salts of penicillin by reason of their relative insolubility in water and attention was focused almost entirely on those that were water-soluble. The suspension of a water-soluble salt of penicillin in a *milieu* of oil and wax has been shown to effectively slow the release of penicillin from the site of its injection into the circulation, and the use of large particle sized of penicillin crystals[1] has further retarded the rate at which penicillin is dissipated from the oil and wax reservoir. The possability of suspending a penicillin salt, relatively insoluble in water, in an oil phase and thus obtain an even slower release of penicillin from the injection site, has only recently been investigated.[7] Procaine-penicillin is the first of these salts to be submitted for clinical evaluation and it is the purpose of this paper to report preliminary observations on the use of procaine penicillin G suspended in oil.*

Material Used. Procaine and penicillin G are combined in equimolecular quantities to form a crystalline salt relatively insoluble in water (less than 0.7% at 28° C.). The particle size of the crystals of this salt is controlled so that at least 50% of the crystals are more than 50 microns in length. The theoretical

* The authors wish to thank Dr. N. P. Sullivan, of Eli Lilly and Company, for kindly supplying procaine penicillin G in oil (Duracillin, Lot No. 8169–P45724 and Lot No. 8044–P45573) for this study and for making available preliminary information pertaining to this preparation.

potency of this preparation should be 1040 units per mg., but potency by test is 940 units per mg. Not less than 90% of the penicillin in this preparation is penicillin G and approximately 41.5% of the weight of this salt of penicillin is procaine. About 7000 units of this salt of penicillin can be dissolved in 1 cc. of water.

Procaine penicillin crystals, such as described above, were suspended in both cottonseed and sesame oils so that each cc. of material contained 300,000 units of penicillin and 125 mg. of procaine. Although both of these preparations were employed in this investigation, the latter proved to be superior by reason of greater stability and fluidity. Whereas the suspension in cottonseed oil must be injected through a No. 18 gauge needle, the sesame oil suspension can be injected through a No. 21 gauge needle. Since the penicillin settles out of either suspension on standing vigorous shaking is required to restore the homogeneity of the preparation. Slight warming facilitates injection of the material through a No. 21 needle.

Methods Employed. Blood specimens for penicillin assays were drawn, with sterile technique, into citrate solution, transferred to graduated centrifuge tubes, centrifuged and the plasma pipetted off. Plasma samples were immediately refrigerated and transported to the laboratory for assay. Penicillin assays were done by a modified Rammelkamp serial dilution method, using a Group A hemolytic streptococcus as the test organism. Correction for the dilution factor introduced by the use of citrate solution was made in all instances.

Patients Studied. Nine ambulatory patients, free of manifest hepatic, cardiac, and renal disease, who were afebrile and partaking freely of fluids and food, were chosen as control patients for this study.* Each patient received 1 cc. (300,000 units) of procaine penicillin suspended in oil as an intramuscular injection into the left buttock. Massage of the injection site was avoided. Thereafter, at 1, 6, 12, 24, 30, 36, 48, 54, 60 and 72 hours respectively, blood specimens were drawn for penicillin assay. This schedule of sampling was adopted in order that bleedings might be done at 9 A.M., 3 P.M., and 9 P.M., times which interfered least with hospital routine.

Eleven consecutive patients admitted to the hospital with lobar pneumonia, as determined by history, physical examination, Roentgen ray examination and laboratory findings, were treated with 1 cc. (300,000 units) of procaine penicillin administered as a single intramuscular injection into the left buttock. No additional therapy in the form of sulfonamides or penicillin was given, until such therapy was indicated by an adverse clinical course. The blood sampling schedule on these patients conformed to that outlined above to the extent that specimens were drawn at 9 A.M., 3 P.M., and 9 P.M. Since, however, patients received the injection of procaine penicillin immediately upon entrance to the hospital, the hours at which penicillin assays were done with reference to this injection do not conform with those of the control group. This program resulted in obtaining information regarding plasma concentrations at intervals not studied in the control group.

The next 12 patients admitted with the diagnosis of pneumonia, were given a single intramuscular injection of 2 cc. (600,000 units) of procaine penicillin into the left buttock without massage of the site. No additional therapy was given until the necessity of it was indicated by the patient's clinical course.

Results. Table 1 presents the blood concentrations of penicillin following the administration of 300,000 units of procaine penicillin to 9 control patients. With the exception of patient J. B., who showed assayable quantities for 30 hours, the penicillin concentrations fell below 0.039 unit per cc. of plasma some time between 12

* Clinical facilities were given us for this study by the following Chiefs of Service: Drs. Frieda Baumann, R. S. Boles, T. M. Durant, H. L. Goldburgh, D. W. Kramer, D. N. Kremer, W. G. Leaman, Jr., T. G. Schnabel, and C. M. Thompson.

and 24 hours after the injection. One hour after the injection, the penicillin plasma concentrations ranged from 0.337 to 2.73 and thereafter declined rather regularly to values lower than could be detected by the method of assay used.

In Table 2 are shown the plasma concentrations that resulted from the injection of 300,000 units of procaine penicillin suspended in oil into patients suffering from pneumonia. Penicillin was detectable for less than 16 hours, less than

TABLE I.

Plasma Concentrations Following 300,000 Units Procaine Penicillin in Oil

CONTROL PATIENTS*

Hours After Injection**	LW 27/M***	CW 62/M	JB 55/M	LE 49/M	JM 18/M	WP 40/M	BM 24/M	CT 31/M	RW 15/M	Average
1	2.73	1.97	0.337	2.05	1.36	0.908	0.908	1.81	1.81	1.54
6	0.678	1.39	0.690	1.33	0.452	0.451	0.908	0.228	0.453	0.729
12	0.043	0.043	0.341	o	0.113	0.042	0.084	0.057	0.042	0.0873
	****									
24	o	o	0.085	o	o	o	o	o	o	o
30	o	o	0.042	o	o	o	o	o	o	o
36	o	o	o	o	o	o	o	o	o	o
48	o	o	o	o	o	o	o	o	o	o
54	o	o	o	o	o	o	o	o	o	o
60	o	o	o	o	o	o	o	o	o	o
72	o	o	o	o	o	o	o	o	o	o

*All of these patients were ambulatory.

**Injection was made routinely into left buttock without massage of the site.

***Age/Sex.

****Concentrations less than 0.039 units/cc. have been regarded as zero.

TABLE II.

Plasma Concentration Following 300,000 Units Procaine Penicillin in Oil

PNEUMONIA PATIENTS

Hours	MK* 33/F**	Hours	EF 39/F	Hours	LH 22/F	Hours	LR 19/M	Hours	WL 37/M	Hours	AC 43/M	Hours	FW 35/M	Hours	JH 69/M	Hours	TM 77/F	Hours	IJ 69/M
1	2.72	1	0.34	9	0.338	16	o	11	0.680	20	0.453	12	0.683	13	0.340	10	0.34	4	1.82
7	0.342	6	0.244	21	0.043	22	o	17	0.340	26	0.228	18	0.225	19	0.227	16	0.341	16	0.113
	***																		
19	o	18	0.113	27	o	28	o	23	0.227	32	0.171	24	0.227	24	0.170	21	0.34	22	0.058
25	o	24	0.113	33	o	40	o	35	0.113	44	0.057	36	0.056	36	o	34	0.17	28	0.057
31	o	30	0.057	45	o	46	o	41	0.056	50	o	42	o	43	o	40	0.114	40	o
43	o	42	o	51	o	52	o	47	o	56	o	57	o	49	o	46	0.134	46	o
49	o	48	o	57	o	64	o	59	o	68	o	60	o	61	o	58	0.057	52	o
54	o	54	o	69	o	70	o	65	o	74	o	66	o	67	o	64	0.113	64	o
67	o	66	o	76	o	76	o	70	o			72	o	73	o	70	0.113	70	o
73	o	72	o																

* This patient was later shown to have tuberculosis.

** Age/Sex.

*** Concentrations less than 0.039 units/cc. have been regarded as zero.

19 hours, and slightly more than 21 hours in patients L. R., M. K., and L. H. respectively. The remaining 7 patients showed penicillin concentrations above 0.039 unit per cc. for periods varying from 24 to 70 hours.

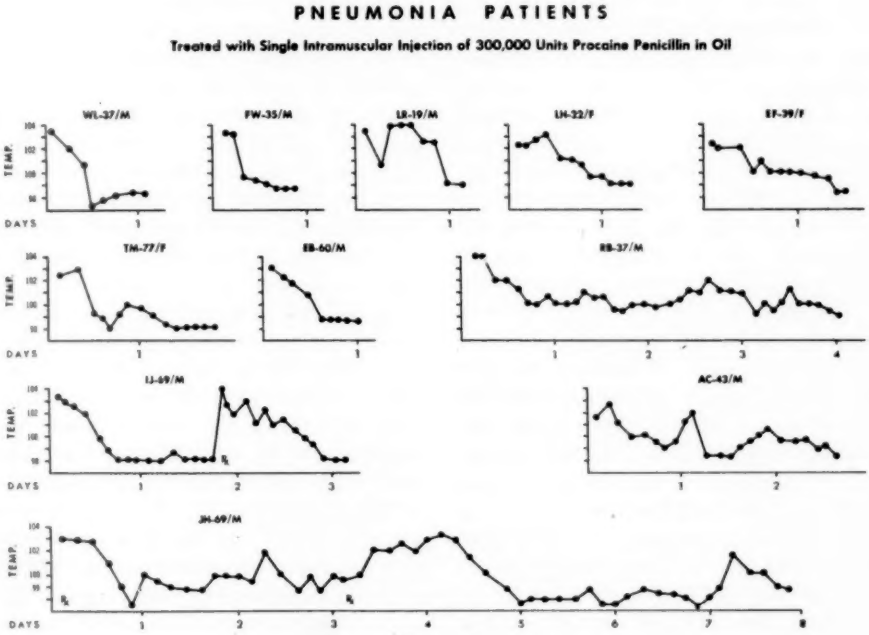

CHART 1

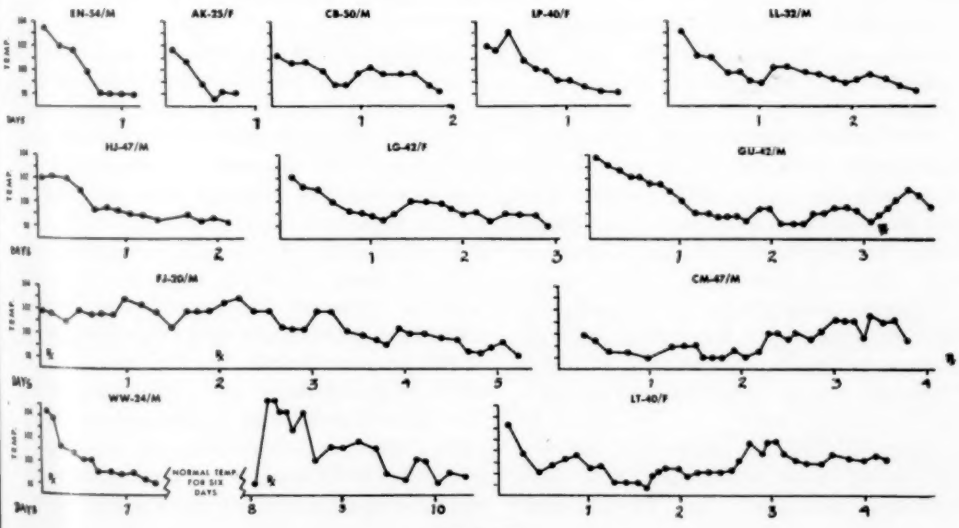

CHART 2

Temperature is one objective criterion by which to judge the response of pneumonia to treatment. In Chart 1 are presented the temperature records of pneumonia patients treated with a single injection of 300,000 units of procaine penicillin suspended in oil. All of these patients responded with a fall in temperature and commensurate clinical improvement and 8 patients went on to complete recovery without further treatment. One patient, R. B., recovered without additional therapy but the response was unsatisfactory and 2 patients, I. J. and J. H., relapsed and required supplementary penicillin therapy.

Chart 2 shows the temperature curves of 12 patients, each of whom received a single intramuscular injection of 600,000 units of procaine penicillin. Eight patients responded satisfactorily without additional therapy. A single patient did not respond to treatment but the fever fell by lysis after secondary procaine penicillin treatment (F. J.). Two patients responded but had return of fever which occasioned second injections of procaine penicillin (C. M. and L. T.), and 1 patient (W. W.) frankly relapsed after 8 days but responded promptly to routine penicillin therapy, 37,500 units intramuscularly every 3 hours.

Discussion. Procaine penicillin in oil, administered intramuscularly into the buttocks of 9 ambulatory and afebrile subjects, resulted in penicillin plasma concentrations above 0.039 unit per cc. for longer than 12 hours in all but 1 patient (L. E.). This patient and one who showed assayable concentrations for 30 hours (J. B.) represent the widest variations in this group. In 10 pneumonia patients, 1 of whom was later shown to have tuberculosis as well, plasma penicillin concentrations above 0.039 unit per cc. were observed for periods varying between 7 to 70 hours. It is suggested that the patients in the control group were ambulatory and well-hydrated and that this circumstance favored the more rapid elimination of penicillin from the reservoir at the site of injection. On the other hand, the acute illness of the pneumonia patients undoubtedly contributed to the prolongation of detectable penicillin levels in the blood. Variability in both the duration and the height of penicillin concentrations following the injections of penicillin in beeswax and peanut oil has been previously observed and related to activity.[3] The long duration of significant concentrations in the case of patient T. M. (age 77 years) is almost certainly related to renal impairment due to age.[6]

Although the group of patients here reported is small, it is of interest to observe that the duration of levels of penicillin above 0.039 unit per cc. for longer than 12 hours in the control group compares favorably with results that have been obtained with penicillin suspended in beeswax and oil at the same level of dosage. Kirby and his associates[3] found that following administration of 300,000 units of penicillin in beeswax and oil they failed to demonstrate penicillin in the plasma beyond 12 hours in 37 of 54 patients (69%). The prolongation of significant levels in the group of pneumonia patients is certainly equal to that reported for penicillin suspended in beeswax and oil.[4,5]

The duration of penicillin in the blood is not the only consideration, however, and if the prolonging effect of procaine penicillin suspended in oil proves equal to that of penicillin suspended in peanut oil and beeswax, the factor of pain incident to the injections may prove to be the more important. Although severe allergic manifestations and actual abscess formation are relatively uncommon, pain and discomfort are very common following use of the beeswax preparation,[1] particularly when repeated doses are administered.[2] The freedom from pain at the site of injection and the lack of systemic toxicity in a group of 50 patients has been gratifying. A single patient, having a history of

food allergy, developed urticaria the day following the single injection. It has not been determined whether the procaine in combination with penicillin still exerts its anesthetic action, but this is being investigated.

In this connection it should be observed that the presence of significant amounts of procaine in procaine penicillin, 125 mg. per 300,000 units of penicillin, suggests the possibility of either aggravating a previously existent sensitivity or establishing a procaine sensitivity. Conclusions on this point must await extensive clinical usage of the material.

In this preliminary investigation of the use of procaine penicillin suspended in oil in the treatment of pneumonia, no effort was made to establish a single injection of the material as the routine therapy of pneumonia, but rather to investigate the duration of activity of a single large dose of penicillin by measuring penicillin in the blood down to a level which is accepted as having therapeutic significance with regard to the organisms ordinarily associated with bacterial pneumonia, and also by determining the actual duration of antibacterial effect or suppressive effect upon an acute infection amenable to penicillin therapy, bacterial pneumonia.

Attention has already been called to the penicillin plasma concentrations in Table 2, but it is of interest to correlate these concentrations with the temperature curves in Chart 1. In no instance was there a recrudescence of fever while a level of penicillin above 0.039 unit per cc. was being maintained (patients W. L., F. W., L. R., L. H., E. F., T. M., I. J., J. H. and A. C.). The abrupt rise in temperature of I. J. suggests, therefore, the termination of the suppressive antibacterial action of the previously administered penicillin. Patients A. C. and J. H. show rises of temperature almost immediately after assayable quantities of penicillin disappeared from the blood, but in the first case the patient's immunologic defenses permitted recovery without additional therapy, whereas this was required in the second case. Penicillin assays were not done on the group of patients who received 600,000 units of penicillin as a single intramuscular injection, but on the basis of the correlation shown above between disappearance of penicillin from the plasma and recrudescence of fever, the recurrence of temperature (Chart 2) in patients G. U., C. M. and L. T. on the 3rd day after treatment is highly suggestive that the antibacterial effect of penicillin had run its course. On the basis of both penicillin assays in the blood and therapeutic response of bacterial pneumonias to a single injection of 300,000 and 600,000 units of procaine penicillin respectively, it is implied that an average period of therapeutic efficacy is 24 to 48 hours. Investigations with intramuscular injections of procaine penicillin at intervals of 24 and 48 hours are in progress and will be the subject of another communication.

Summary and Conclusions. 1. No local pain or systemic toxicity have been observed in 50 patients to whom procaine penicillin G has been administered.

2. The duration of penicillin plasma concentrations above 0.039 unit per cc. varied from 6 to 30 hours in a group of 9 ambulatory control patients. In a group of 10 pneumonia patients it varied from 7 to 70 hours. The averages in the 2 groups were 12 hours and 33 hours respectively.

3. Eighteen of 23 patients suffering from bacterial pneumonia recovered without complication following a single intramuscular injection of procaine penicillin G in oil. Two patients relapsed under this therapy and 3 failed to respond.

4. On the basis of these preliminary data, it appears that a single injection of 300,000 units of procaine penicillin will, in the average patient, give assayable plasma concentrations of penicillin, or a suppressive antibacterial action, for at least 24 hours.

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MYOCARDIAL CHANGES IN FATAL DIPHTHERIA

A Summary of Observations in 221 Cases

By Ira Gore, M.D.
PATHOLOGIST
WASHINGTON, D. C.

(From the Veteran's Administration, Central Laboratory, Army Institute of Pathology)

Despite the continuous downward trend of the incidence of diphtheria in the United States since the practice of immunization became widespread, the disease has not been conquered. Edsall[6] pointed out that the tremendously decreased carrier rate in artificially immunized communities has been associated with a rising incidence of susceptibility as indicated by the Schick test. Furthermore, the reduced exposure to diphtheria bacilli with the concurrent loss of its stimulating effect has contributed to the more rapid waning of artificially induced immunity.[6] Such circumstances set the stage for a widespread reappearance of the disease should the virulent organisms be introduced into the population. Considering the present incidence of diphtheria in several of the European countries[20,21] (Table 1, A) the possibility is far from remote. Though the peak appears to have been passed in many of these countries, even the most recent figures are considerably above the prewar annual median incidence. Liebow and Bumstead[13] have traced localized outbreaks in U. S. Army General Hospitals to the introduction into the wards of unrecognized cutaneous diphtheria. A recent report[14] noted a significantly greater number of cases of diphtheria during 1946 in both New York City and New York State than during similar periods of 1945. The introduction of large numbers of U. S. troops into areas where the endemic diphtheria rate was high resulted in a striking increase of the incidence of that disease among them (Table 1, B). The situation among occupying troops has not changed since the end of the conflict.[5] Though no studies have been reported, it is highly probable that the number of

Table 1

A. *Cases of Diphtheria Reported in European Countries**

Country	Pre-war annual median	Number of cases reported annually 1943	1944	1945
Belgium	2,089	16,072	6,295	5,808
France	19,389	46,539	40,430	37,262
Germany	78,452	241,609	207,392	.. *
Netherlands	3,967	56,825	60,226	78,098
Norway	968	22,787	13,547	7,466
Sweden	1,484	2,496	6,040	4,615

B. *Annual Incidence of Diphtheria Among U. S. Troops (Per 100,000)*†

	1942‡	1943	1944	1945
Total Army	3.76	9.72	21.44	53.81
Troops within the U. S.	2.52	3.54	6.83	15.66
Troops outside U. S.	9.39	30.01	38.20	81.19

(Figures from Medical Statistics Division, S. G. O.)

* Complete data not available. Compiled from figures in References 2 and 3, UNRRA Epidemiological Information Bulletins, Vols. **1** and **2**, 1945, 1946, and Public Health Reports Vol. **61**, No. 7, 2–15–46.

† All rates shown are preliminary.

‡ The rates shown for the year 1942 are based on sample tabulations of individual medical records, while those for the last 3 years are based on the Weekly Statistical Health Report, which source probably overstates the actual incidence.

carriers among returned military personnel is greater than among the general populace.

In view of these developments and their ominous potentialities it is thought appropriate and timely to report a study of that sequela of diphtheria which is most often lethal, myocarditis. During a more inclusive review of the subject of myocarditis[10] a large number of cases were found to be associated with diphtheria. The present study is based on a review of the autopsy records and slides of 221 fatal cases of diphtheria accumulated at the Army Institute of Pathology. Material from the heart was available for review in 205 of these. The presence or absence of myocardial disease was determined in each case from the few routinely taken sections of the myocardium. In many instances fat stains, Gram stains, or Giemsa stains of involved heart muscle were prepared. For reasons which will become apparent in the text it was also considered important to ascertain the presence or absence of peripheral neuritis. The latter was determined solely from the clinical records because the available material was inadequate for pathologic study. Though there is a disparity in the method of diagnosis, the clinical report of neuritis is regarded as a sensitive and reliable index of its presence since it is a common experience for pathologists to find a paucity of morphologic changes in nerves to explain rather striking symptoms of nerve injury. The number of instances of myocarditis and peripheral neuritis encountered is listed in Table 2.

TABLE 2.—SEQUELÆ ENCOUNTERED IN 205 FATAL CASES OF DIPHTHERIA

	Cases	%
Myocarditis alone	99	48.3
Myocarditis with neuritis*	44	21.5
Neuritis* alone	6	2.9
No sequelæ	56	27.3
Total	205	100.0

* The presence or absence of neuritis was determined solely from the clinical records, whereas the diagnosis of myocarditis was based on the pathologic findings.

Age. The age distribution of the patients of this series is listed in Table 3. Exclusive of those younger than 18, it was found that 51% of the series was made up of individuals of under 25 years of age, a group constituting 43% of the Army, which was the source of the bulk of this material. The greater susceptibility of younger persons to diphtheria has long been recognized. However, Geiger[9] has commented upon a noticeable shift in incidence from early age groups to those of 30 years and over; an observation easily explained by the conditions discussed in the introductory paragraphs.

TABLE 3.—AGE DISTRIBUTION OF PATIENTS WITH FATAL DIPHTHERIA

Age group	Cases	%
0–9	22	10.8
10–19	31	15.3
20–29	95	46.8
30–39	48	23.6
40 and over	7	3.4
Unknown	18	..
Total	221	100.0

Color. There was no significant difference between diphtheria rates for white and Negro as far as can be determined from the fatal cases studied. Seventeen, (7.2%) of the patients were non-white, a figure comparable to the proportion (approximately 10%) in the whole army.

Sex. The number of women was far too few to permit analysis of possible sex differences.

Clinical Observations. *Duration of Illness.* The reported duration of illness varied from 1 to 182 days and is summarized in Table 4. Three-fourths of the deaths occurred from 6 to 24 days after the onset of diphtheria. The incidence of

TABLE 4.—LENGTH OF ILLNESS IN 221 CASES OF FATAL DIPHTHERIA

Days	Patients	%
0–1	5	2.8
2–5	27	15.2
6–12	59	33.1
13–24	48	27.0
25–45	18	10.1
46–75	18	10.1
Over 75	3	1.7
Unknown	43	..
Total	221	100.0

both myocarditis and neuritis rose progressively with the duration of illness before death, a relation shown graphically in Chart 1. Age had no apparent relation to the development of either sequela. Myocarditis was present in 70% of the patients who died between the 6th and 24th day, the average duration of illness in these individuals being 14 days. Peripheral neuritis, on the other hand, appeared to develop somewhat later, generally between the 13th and 45th days, the average duration of illness before its onset (among patients in whom symptoms of this condition were reported to have appeared before death) being about 20 days.

TABLE 5.—MANIFESTATIONS OF CARDIAC DYSFUNCTION IN 143 FATAL CASES OF DIPHTHERITIC MYOCARDITIS

	Patients	%
Shock*	74	51.4
Cyanosis	56	38.9
Congestive failure†	49	34.0
Tachycardia	47	32.6
Dyspnea	32	22.2
Substernal pain or oppression	16	11.1
Arrhythmia	11	7.6
Orthopnea	4	2.8
None	9	6.2

* Includes hypotension, weak thready pulse, and imperceptible heart action.

† Includes venous distension, pulmonary edema, hydrothorax, and painful, swollen liver.

Cardiac Manifestations. Signs and symptoms (Table 5) indicative of seriously impaired heart function were present in most of the patients studied, but in a large proportion the presence of myocarditis had not been suspected before death. In many instances, however, the brevity of the interval between the cardiac manifestations and death precluded such recognition. Furthermore, in one-third of the patients the local lesion had resolved and the fever had subsided for a varying interval prior to the onset of cardiac dysfunction. In Chart 2 this latent period has been designated as the "deceptive interval of apparent improvement." Electrocardiograms were made on 52 patients (presumably because of the presence of cardiac symptoms) and abnormalities were present in all of them (Table 6).

TABLE 6.—ELECTROCARDIOGRAPHIC ABNORMALITIES IN 52 PATIENTS WITH DIPHTHERITIC MYOCARDITIS

Delayed conduction	19
Other conduction disturbances	14
"Myocardial damage"	18
Extrasystoles	4
Ventricular tachycardia	4
Tachycardia (type unspecified)	2
Abnormality not specified	9

Neuritis. Neuritis, symptoms of which were reported for 50 patients, involved the cranial nerves first and most frequently. The manifestations included difficulty in swallowing, regurgitation of food, nasal voice, interference with phonation, and visual disturbances. Evidence of involvement of the nerves to the extremities as indicated by pain, weakness, absent reflexes, or paresis was observed in 26 patients. In no instance were the nerves to the limbs affected in the absence of cranial nerve damage. Among the patients with neuritis, those dying between the 6th and 29th day of illness had neurologic manifestations largely limited to the cranial nerves. Patients surviving beyond 24 days included most of those with clinical evidence of involvement of the nerves to the arms or legs. Therefore, both from the number of cases and the duration of illness among them, it would seem that cranial nerves are more susceptible than peripheral nerves.

Complications. Pneumonia, usually of the patchy variety, was by far the most frequent complicating illness. It was present in 50% of the patients who died within 5 days of the onset of diphtheria, and in 86% of those who lived longer than 45 days. Altogether pneumonia occurred in 134 of the 221 fatal cases. The pneumonic process was associated with pericarditis in 5 patients and bacteremia in 4; in a single instance it was associated with Ludwig's angina.

Pathologic Alterations In the Heart. *Gross Observations.* Although many of the autopsies were done under wartime field conditions by men without special pathologic training, gross cardiac abnormalities were reported in 71% of the cases. Dilatation of the chambers, flaccidity, pallor,

and "streakiness" of the myocardium, noted singly or in combination, were the changes most frequently reported. In a few cases of prolonged illness small focal areas of scarring were observed. Occasionally, small mural thrombi were found in the left ventricle. Relating these gross features with the microscopic findings described below revealed a direct correlation between them; when the latter indicated severe involvement, gross abnormalities were almost invariably reported (Table 7).

TABLE 7.—GROSS CARDIAC ABNORMALITY IN RELATION TO ESTIMATED DEGREE OF MYOCARDITIS

Myocarditis	Number of patients	% showing abnormality
Absent	59	50.8
Slight	17	58.8
Moderate	85	84.7
Severe	34	97.1
Total	195	

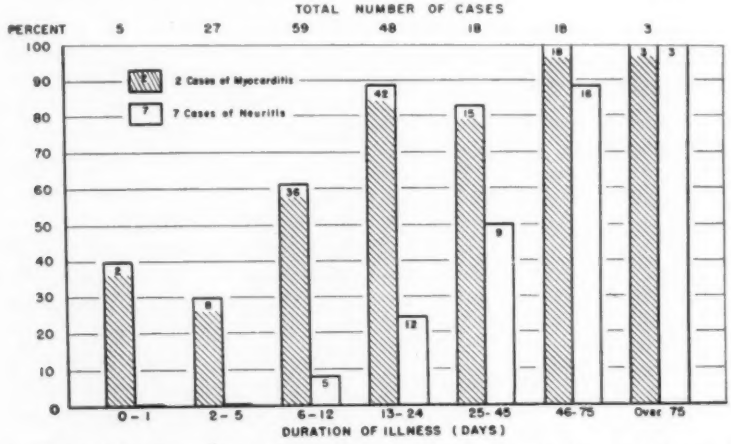

CHART 1.—The relation of myocarditis and peripheral neuritis to the duration of fatal diptheria.

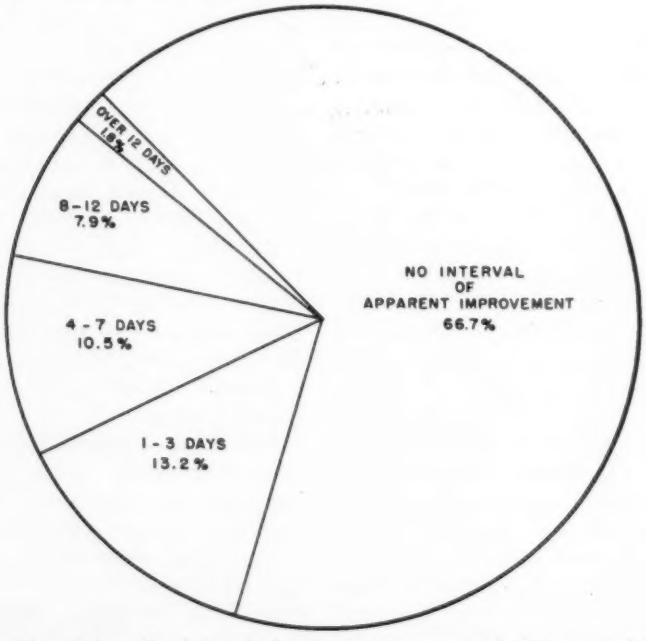

CHART 2.—Duration of deceptive interval of apparent improvement in 221 cases of fatal diphtheria

Heart Weight. The weight of the heart was found to be increased in some cases in the absence of a history of hypertension, kidney disease, heart disease, heart valvular lesion, or other condition known to be associated with myocardial hypertrophy. Sufficient data were available in 134 cases to utilize the tables of Smith[18] as a criterion of such an increase (Table 8). Increased heart weight was present in more than one-half of the 93 patients with myocarditis. More impressively, 86% of the enlarged hearts were the seat of myocarditis and, as is evident in the table, the severity of that process seemed to bear a direct relation to the degree of cardiac enlargement as well as to its presence.

TABLE 8.—DEGREE OF MYOCARDITIS IN RELATION TO MEAN EXCESS OF HEART WEIGHT OVER NORMAL*

Degree of myocarditis	Number of cases	Mean excess of heart weight (gms.)
None	24	32
Slight	7	52
Moderate	46	84
Severe	17	104

* Heart weight computed in relation to body weight in accordance with tables of H. L. Smith, American Heart Journal, Vol. **4**, 1928.

Microscopic Observations. As has been already intimated, the extent of the myocardial changes varied considerably from severe involvement with large and multiple areas of muscle damage to mild changes with only occasional small foci of involvement. In all cases, however, the qualitative changes were identical. Organisms were not demonstrable microscopically in any of the involved hearts. The sequential changes in the myocardial lesion from the onset of diphtheria to the fatal outcome are characteristic, and by studying them one may make a fair estimate of the duration of illness. When death occurs early in the course of the disease, changes are inconspicuous, consisting of mild interstitial edema and cloudy swelling of the muscle fibers. The cells of the interstitium are hyperplastic and plump but not increased in number, and there is no inflammatory cellular infiltrate.

If the disease has gone on for a week, fairly conspicuous lesions of the cardiac muscle become apparent. Diffusely scattered foci of hyaline and granular degeneration involve segments of the muscle fibers which frequently are attenuated, severely fragmented, or ruptured. A mild, stromal inflammatory response is often observed intrafascicularly adjacent to injured muscle, but the large interfascicular stromal septa are relatively unaffected. The cellular reaction is believed to be in the nature of a reparative response, since it takes place only in the presence of damaged muscle, whereas muscle damage may occur without significantly increased stromal cellularity. The infiltrate consists largely of histiocytic cells, many of which have the characteristics of the so-called "myocyte" of Anitschkow,[7] indicating that they have arisen *in situ*. A small number of lymphocytes and plasma cells are present; polymorphonuclear leukocytes, eosinophils, mast cells, and others are even more infrequent and seem not to play an essential rôle in the process. The muscle lesion, as has been reported by other observers,[3] involves especially the inner portion of the myocardium and the papillary muscles. There is no apparent predilection for any particular one of the heart chambers, nor does the conduction bundle appear to be more or less susceptible than other portions of the musculature.

During the second week, plasma cells and lymphocytes become more numerous although the histiocyte remains the predominant cell type (Fig. 1). Hyaline and granular degeneration is still extensive, with the addition of small foci in which muscle fibers have disappeared, leaving a loose stromal net, moderately infiltrated with macrophages and small round cells. A few macrophages contain brown, granular pigment.

Myolysis, disintegration, or "dropping out" of the degenerated muscle fibers becomes conspicuous during the third week. Grossly, the myocardium appears "streaky," and microscopically the heart

appears "moth-eaten" because of the irregular foci of parenchymal loss. In such foci there is little more than the preexistent stroma with hyperplastic cells containing an infiltrate of histiocytes, plasma cells, and lymphocytes. Areas of muscle loss, show marked hyperplasia and abortive regeneration (Fig. 2). If such an area happens to be immediately adjacent to the endocardium, the alterations in that layer may lead to the formation of a mural thrombus.[5a,22]

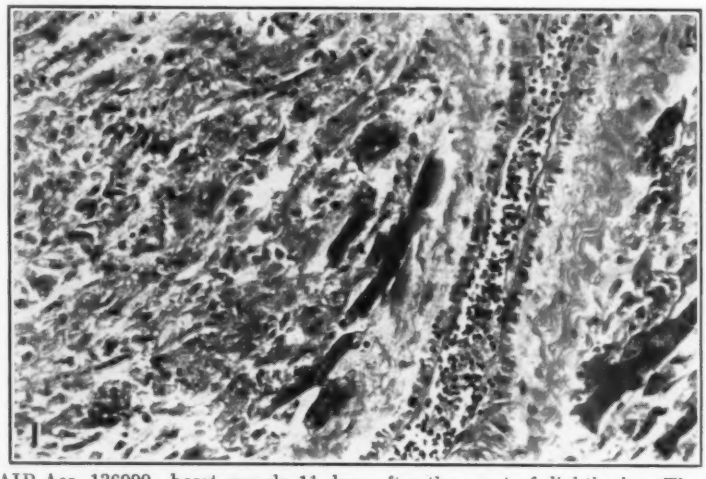

FIG. 1.—AIP Acc. 136990—heart muscle 11 days after the onset of diphtheria. The degenerated fibers have lost their form and are represented by granular amorphous material. The cellular inflammatory reaction is scant at this stage. (Neg. 93109 × 230.)

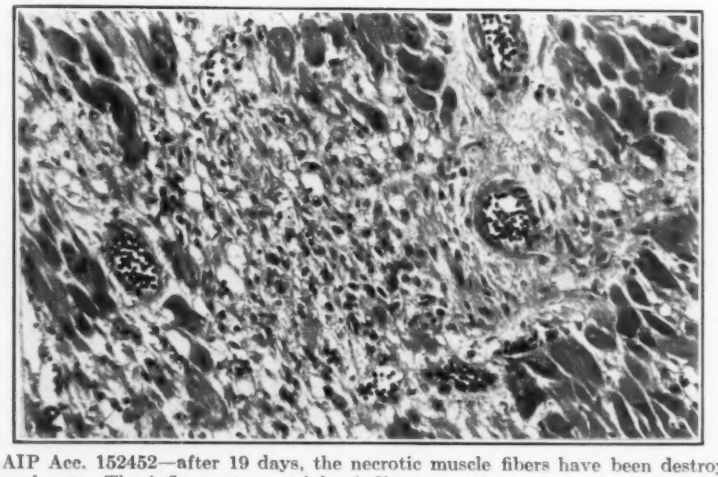

FIG. 2.—AIP Acc. 152452—after 19 days, the necrotic muscle fibers have been destroyed leaving a loose stromal net. The inflammatory cellular infiltrate consists principally of large mononuclear cells, lymphocytes and plasma cells. (Neg. 93107 × 230.)

hyaline degeneration are much less prominent than at earlier stages. By the end of the third week the residual muscle fibers, especially those bordering areas of

After the third week there is progressive fibrosis replacing the lost muscle. Adjacent to these areas of scarring, myofibrillar hypertrophy and nuclear enlarge-

ment, distortion, and hyperchromatism are striking (Figs. 3 and 4). The cellular infiltrate steadily decreases without any change in its differential pattern. Focal hyaline degeneration becomes increasingly rare; however, it was still noticeable in one case in which death was delayed for 5 weeks. As would be expected, the amount of myocardial damage is less in instances of prolonged survival, but in many of these there are neurologic sequelæ which contribute to the fatal outcome.

Fatty degeneration of the myocardium, present in more than half of the 60 cases studied for this feature by Councilman, Mallory, and Pearce,[5] was also an inconstant finding in Warthin's[22] series and in this study. In many cases large numbers of tiny fat droplets are found in muscle fibers which routine stains show to

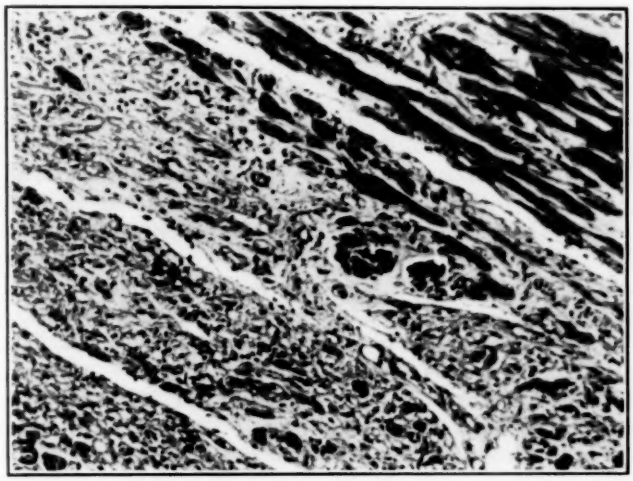

FIG. 3.—AIP Acc. 143672—after 35 days the muscle lesion in the heart shows early fibrosis. A few deeply staining, necrotic fibers may still be seen. (Neg. 93106 × 160.)

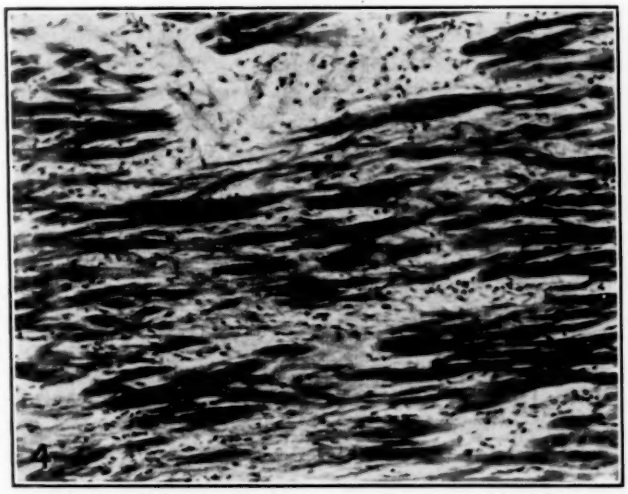

FIG. 4.—AIP Acc. 140532—fibrosis is complete on the 55th day. Note the large, deeply staining, bizarre nuclei of muscle fibers bordering the fibrous areas. (Neg. 93105 × 160.)

have degenerated. In an occasional instance, particularly if death occurs early, fat stains delineate foci of degeneration not apparent in routinely stained sections.

Morphologic changes were not seen in the cardiac nerves included in the sections of myocardium studied.

In brief, it can be said that diphtheritic infection produces a varying degree of parenchymal damage, manifested as segmental hyaline, granular and fatty degeneration of the muscle fibers. The secondary inflammatory response of the stroma appears at first to consist largely of locally developed histiocytes, but it is soon augmented by an influx of plasma cells and lymphocytes. Degenerated muscle is destroyed, leaving gaps and defects in the myocardium, which, if the length of survival permits, heal by fibrosis. The muscle shows only abortive regeneration. Statements by Anitschkow, Heller, and Warthin[22] to the effect that there is true muscle degeneration were based on Anitschkow's belief in the myogenic function of the "myocyte," a cell now generally regarded as having a histiocytic function.

Discussion. The figures from clinical studies show a much lower incidence of myocarditis than was found in this postmortem study. Hoyne and Welford[11] reported 496 complicated by myocarditis among 4671 (10.6%) consecutive cases of diphtheria. Kay and Livingood[12] found 7 among 141 (5%) cases of cutaneous diphtheria. The electrocardiograph enabled Burkhardt *et al.*,[2] to find myocarditis more frequently, since they reported abnormalities in 28 of 140 (20%) consecutive cases of diphtheria. It may be inferred that other methods of clinical diagnosis were less sensitive and were not as accurate a gauge of the true incidence of myocarditis. The presence of abnormalities in each of the cases of this series for which electrocardiograms were made is indicative only of a high degree of selectivity in choosing the patients to be tested. Such a perfect correlation could not have been expected had electrocardiograms been taken in all cases in the series. Demonstrable myocardial damage in each of these hearts, nevertheless, is evidence of the value of electrocardiographic studies. Hoyne and Welford[11] found a mortality of 62% in the presence of myocarditis in their series, a figure more than five times the 11.6% overall fatality rate in their 4671 consecutive cases of diphtheria. Such a selective mortality serves to explain why myocarditis is found more frequently in post-mortem than in clinical studies.

The modern concept of the pathogenesis of the heart lesion in diphtheria stems from Loeffler's report (1884) that the organism does not extend beyond the local lesion. A few years later, Roux and Yersin isolated the toxin which produces the remote tissue changes. The lesions produced experimentally, as well as those observed in autopsies of human diphtheria, support the statement of Topley and Wilson:[23] "There can be little doubt that the direct action of diphtheria toxin on the heart muscle is one of the most important causes of death." The toxin which is quickly absorbed into the blood rapidly becomes fixed by the tissues, forming a combination which is not broken by the administration of antitoxin.[15] Both functional and morphologic cardiac effects appear early.[23] These characteristics provide the logical foundation for the universally accepted principle of administering antitoxin promptly and in adequate dosage. In this series 132 patients had received diphtheria antitoxin but the available data are inadequate for an analysis of the efficacy of early administration of antitoxin, and, in any case, this point has been so well substantiated in the past that further corroboration is superfluous. Since it has been reported that sulfonamide,[8] serum,[4] or other drug[1,24] sensitivities are capable at times of producing demonstrable myocardial changes, it is interesting that neither the severity, incidence, nor character of the myocarditis was altered by penicillin (98 cases), sulfonamides (86 cases), or serum reactions (9 cases). Though these agents would

have no antitoxic value, neither was there evidence that they contributed to the cardiopathic action of diphtheria toxin.

Neuropathic effects are also a well known sequel of diphtheritic infection. The clinical manifestations are so striking that at one time, as reported in Warthin's[22] review, myocardial damage was regarded as secondary to primary nerve injury. Though this concept has been largely abandoned, its occasional resurrection is sufficient reason for comparing myocardial and neuritic effects among the patients of this series. It is to be noted that the numerical and chronologic differences between diphtheritic myocarditis and neuritis depicted in Chart 1 are incompatible with any hypothesis which ascribes morphologic cardiac changes to the nerve injury. Furthermore, other conditions which at times affect the nerves supplying the heart do not duplicate the myocardial changes observed in diphtheria. Among such conditions are poliomyelitis, the Guillain-Barré syndrome and a variety of tumors arising from, or metastasizing in, the region extending from the base of the skull to the upper mediastinum. It seems reasonable to conclude that the myocarditis and neuritis occurring in diphtheria are not causally related to each other.

Although the myocardial changes have been frequently referred to as myocarditis in this report, it should be pointed out that the various authors[16] do not regard a primarily parenchymatous lesion as a true inflammation. However, since inflammation is a biologic response to injury, it seems too fine a distinction to segregate conditions in which injury is morphologically evident from those with more subtle types of cell damage. Histochemical techniques developed within the last few years may provide the means of demonstrating some of these more subtle evidences of cell injury, thus breaking down the artificial distinction between interstitial and parenchymatous inflammation.

The relationship between the myocarditic process and cardiac enlargement deserves brief comment. In the medical literature hypertrophy of the heart in "isolated" or Fiedler's myocarditis is often regarded as a feature of that disease.[17] However, since it was frequently present in myocarditis associated with a variety of conditions other than Fiedler's myocarditis or diphtheritic myocarditis[10] its specificity can no longer be given serious consideration. The use of the term hypertrophy, too, is not, strictly speaking, accurate. Increased size and weight is a natural consequence of the inflammatory process within the myocardium; the muscle fibers themselves are not hypertrophied.

Clinically, the decéptive interval of improvement prior to the onset of cardiac dysfunction frequently observed in diphtheria[15,19] is a characteristic deserving of re-emphasis, especially since it has been found to occur in myocarditis of diverse etiologies.[10] Perhaps, if in diphtheria, myocarditis (or neuritis) were regarded as a sequela to be anticipated as a pharmocologic effect of the toxin, rather than as a complication that develops unexpectedly, a greater proportion of the cases would be recognized early enough to permit adequate remedial measures. Proper therapeutics would recognize the greatly reduced functional capacity of the heart, with the result that the patient's activity would be restricted and great caution observed in the administration of intravenous fluids often and disastrously given in copious quantities to combat a shock-like state which is itself a manifestation of cardiac embarrassment. Proper regard must be given the prolonged convalescence required for healing of the myocardial lesion. Should such measures prove successful in conserving patients with severely damaged hearts, permanent cardiac residua of clinical importance may occasionally result depending upon the quantity of myocardium destroyed. The general denial of permanent clinically significant heart impairment stems from the general failure to save such patients in the past.

Summary. 1. The very high incidence of diphtheria in portions of Europe constitutes a medical hazard to this country under modern conditions of communication.

2. Myocarditis, a frequently lethal sequel, occurred in this series in 143 of 205 (70%) fatal cases of diphtheria. A higher mortality in the presence of myocarditis largely explains why its incidence in postmortem studies is so much greater than in clinical reviews of diphtheria. A certain proportion of missed diagnoses serves as an additional explanation for this difference.

3. As the length of survival from the onset of diphtheria increases the incidence of myocarditis rises. A similar time relation is true for neuritis. However, postdiphtheritic neuritis appears later and is less frequent than myocarditis. The two sequelæ are not related causally to each other. Neither age nor color appear to have any influence upon them.

4. Pathologically, the hearts are dilated, with flabby, pale, or mottled musculature. Frequently the hearts are enlarged, a finding which appears to be related to the presence and degree of myocarditis, and not to myocardial hypertrophy. Microscopically, there is a primary toxic degeneration of the myocardial fibers. The inflammatory response, which culminates in scarring, appears to be secondary to the muscle injury.

5. In one-third of the cases the manifestations of myocarditis appeared at a time when the patient seemed to be well on his way to convalescence. The interval between diphtheria and the onset of cardiac symptoms has been designated the deceptive interval of apparent improvement.

6. The importance of administration of antitoxin early and in adequate quantities is re-emphasized as a measure to prevent myocarditis (and neuritis).

7. The designation of myocarditis (and neuritis) as a sequel of the pharmacologic effect of the toxin rather than as an unexpected complication may contribute to the early and more frequent recognition of myocarditis.

8. A shock-like state following diphtheria must be recognized as a frequent manifestation of myocardial weakness lest the vigorous administration of intravenous fluids to combat it result in a fatal termination.

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STUDIES OF URINARY EXCRETION OF METHIONINE BY NORMALS AND BY PATIENTS HAVING LIVER DISEASE

By James E. Wheeler, M.D.
RESIDENT IN PEDIATRICS
AND
Paul György, M.D.
PROFESSOR OF CLINICAL PEDIATRICS
PHILADELPHIA, PENNSYLVANIA

(From the Nutritional Service of the Department of Pediatrics and the Gastro-intestinal Section of the Medical Clinic, School of Medicine, University of Pennsylvania)

With the increasing use of methionine in the treatment of diseases in which there is liver damage, the need for further study of the metabolism of this essential amino acid is evident. It is particularly important to establish the degree to which ingested synthetic methionine is utilized by the body, especially for comparison of normals with patients having parenchymatous damage of the liver. While there have been earlier studies of this type, they have been hampered by analytical methods of doubtful validity when applied to biological fluids.

This report deals with the urinary excretion of added dietary dlmethionine by normal children and adults as compared with patients of similar age and weight who showed laboratory or clinical evidence of liver disease.

Experimental. Control assays were made on healthy individuals performing routine hospital duties and eating the customary diet served to hospital personnel. Routine hepatic tests, including cephalin cholesterol flocculation, thymol turbidity, thymol flocculation, and serum bilirubin, gave results for these individuals that were within normal limits.

Patients were chosen for study on the basis of liver damage demonstrable clinically and by laboratory tests. Confirmed diagnoses in this group include 1 case of toxic (aminothiazole) hepatitis and 3 cases of portal cirrhosis.

A group of 6 children being studied for chronic, afebrile disease, whose liver function tests indicated no abnormality, served as controls. They remained on the customary hospital diet.

Seven children who were suffering from viral hepatitis were studied. One presented frank viral hepatitis. In the remainder there was demonstrable laboratory evidence of liver damage and minimal clinical signs and symptoms. The diagnosis in this latter group was hepatitis without jaundice.

An attempt was made to keep the diets of controls closely comparable to those of the patients. This was facilitated in 12 of the children by the fact that both groups were served indiscriminately from the same food cart.

Dl-methionine was given in the form of 0.5 gm. tablets of "Meonine." For the children aqueous solutions were prepared from chemically pure crystals of the *dl*-form.*

Urine was collected quantitatively for a control period of 24 hours. Methionine was then given in a single large dose (5 gm. for adults, 3 gm. for children), although it was realized that such administration was unfavorable for maximal utilization. Urine was subsequently collected for three succeeding periods of 24 hours each. Samples were collected in brown bottles containing 20 ml. of 20% HCl, layered with toluene, and stored in a cold room at 4° C.

Assay of Methionine in Urine. The microbiologic assay for methionine developed by Dunn,[4] adapted for use with oxidized peptone media prepared by the method of Lyman, *et al.*,[10] was used for all analyses. The organism used was Lacto-

* Furnished through the courtesy of Wyeth, Incorporated.

bacillus fermentii—36, which utilizes both the d- and l- forms of methionine. Titratable lactic acid produced by a standard growth of the organism served as an index of methionine present.

Urea, the principal growth-inhibitor (to Lactobacilli) found in urine, was removed by treatment of urine with purified urease. Crude urease solution, prepared by extraction of jack bean meal with 15% ethyl alcohol, was dialyzed to remove free amino acids.[2] Urease treatment of urine was carried out according to the technique of Hui Lan Yeh, *et al.*[8] Standard aqueous methionine solutions subjected to the same steps of urease treatment showed recoveries of 100% (+ 10%). It was found in earlier work that sodium chloride inhibited growth of the Lactobacilli used, but under our conditions this effect was negligible up to a concentration of 2% NaCl.

Hydrolysis of urine increases the concentration of methionine found.[8] This effect was confirmed. In normal urines it is improbable that the increase of methionine values found after hydrolysis is due to liberation of methionine from proteins present in the urine; however, in pathologic urines this possibility cannot be excluded. The finding of wide differences between bound and free methionine at intervals following ingestion of a single large oral dose (of Table 3) supports the hypothesis that the increase is due to liberation of methionine from a simple, hydrolyzable, low molecular compound rather than from a protein complex.

Hydrolysis was accomplished by refluxing urine 6 hours in 6N HCl according to a method previously described.[8] Again, standard aqueous methionine solutions subjected to the same treatment showed recoveries of 100% (+ 10%).

It was thought that the increased acidity and browning discoloration (Maillard reaction) produced by autoclaving the assay media might be a source of error. This effect was investigated by assay of Seitz-filtered aqueous standard solutions, urine, and urine with added methionine, in duplicate with the same solutions sterilized by autoclaving 12 minutes at 15 pounds pressure. The results indicate that there was no interference.

TABLE 1.—RESPONSE BY CONTROLS AND BY PATIENTS STUDIED TO HEPATIC TESTS

Patients	Age	Wt. (lbs.)	Diagnosis	Serum bilirubin (mg./100 ml.)	Cephalin chol. flocculation	Thymol turbidity (units)	Thymol flocculation	Colloidal gold	Colloidal red	Remarks
Adults:*										
RT	30	150	Normal	1.3	0	2.5	0	±	±	
LB	37	160	Normal	0.77	0	2.0	0	0	0	
JE	26	180	Normal	0.65	0	0.5	0	0	0	
FH	54	170	Cirrhosis	13.3	4+	2.8	3+	3+	..	Biopsy†
PP	52	150	Cirrhosis	0.33	4+	3.0	0	4+		
JF	58	135	Cirrhosis	0.10	3+	1.0	0	0	..	Biopsy (2 ×)†
BD	13	130	Hepatitis	8.8	4+	5.0	0	2+	..	Diag. porphyrinuria
Children:										
Rh	12	58	Normal	0.32	0	1.0	0	0	0	No bilirubinuria
De	14	65	Normal	0.16	0	0.5	0	0	0	No bilirubinuria
Me	11	72	Normal	0.21	0	1.0	0	0	0	No bilirubinuria
Ho	14	68	Normal	0.42	0	0.5	0	0	0	No bilirubinuria
Dn	15	70	Normal	0.20	0	1.5	0	0	0	No bilirubinuria
Ro	10	52	Normal	0.28	0	1.0	0	0	0	No bilirubinuria
Gu	9	61	Hepatitis	2.13	4+	8.0	4+	4+	4+	Frank jaundice
Ge	15	77	Hepatitis	1.02	3+	0.7	0	±	±	Bilirubinuria
Da	15	67	Hepatitis	1.11	4+	1.5	0	2+	2+	Bilirubinuria
Kr	16	76	Hepatitis	0.67	2+	1.0	0	3+	3+	Bilirubinuria
Mc	15	52	Hepatitis	0.25	0	0.7	0	0	0	Bilirubinuria
Kr	11	50	Hepatitis	0.29	0	0.5	±	0	0	
Ma	14	70	Hepatitis	0.06	0	0.5	0	0	0	

* This individual usually has a slightly elevated serum bilirubin, but repeated liver function tests have given results within normal limits.

† In these cases, the diagnosis was confirmed by needle biopsy of the liver.

Results. Methionine excretion in the control pre-ingestion period, representing the daily basal urinary excretion, is shown in Table 2.

Variations of urinary excretion of dl-methionine after dietary supplement are shown in Figure 1. It will be noted that the amount of dl-methionine found in urine after ingestion of a single dose increased sharply in the first 24-hour post-ingestion period, whereas during the second 24 hours it had returned almost to the control pre-ingestion level. The increased excretion during the first 24 hours represents the portion of ingested dl-methionine which had been absorbed from the gastro-intestinal tract and not retained by the tissues. The greatest amount of excreted dl-methionine found for the first 24-hour period was approximately 15% of the amount administered, while the average found was approximately 10%.

The patients showing signs of marked liver damage both clinically and by hepatic tests (adult abnormals) exhibited pre-ingestion levels which were higher than in the normals. When these data were subjected to statistical analysis by the chi square method, the differences were found to be of marginal significance. Similar observations were also made in the second post-ingestion 24 hours, indicating delay in return to the pre-ingestion level.

Fractional excretion during a single 24-hour period was studied in one fasting normal. Following a single dose of 3 gm. given as "Meonine" tablets urine was fractionally collected at convenient intervals. No attempt to establish diuresis was made. Samples were assayed both after urease treatment and after hydrolysis with HCl. The results, listed in Table 3, show that a considerable portion of dl-methionine is excreted in a combined form.

Discussion. The first reported study of urinary excretion of methionine by normal subjects was that of Albanese,[1] who, using a chemical method, found 247 to 494 mg. per 24-hour period. Homburger[7] employing the same analytical technique, studied methionine excretion by normals, 1 patient with nephrosis, and 2 patients showing evidence of liver damage. He found values of approximately 200 to 400 mg. per 24 hours in the pre-ingestion urines of normals, and values of approximately 200 to 600 mg. per 24 hours in pre-ingestion samples of a patient suffering from hepatolenticular degeneration. He further found an increased excretion by patients showing liver damage given supplementary methionine as compared with normals.

In this study, preliminary analyses based on chemical methods yielded inconsistent results. The method of Albanese,[1] based on the oxidation of methionine by iodine was considered as lacking the necessary specificity for biological fluids. The colorimetric nitroprusside reaction as described by Csonka and Denton[3] was investigated, and values found in normal

TABLE 2.—DAILY BASAL URINARY EXCRETION OF METHIONINE

Normals			Abnormals	
Name	Methionine found mg. per 24 hours		Name	Methionine found mg. per 24 hours
Adults:				
RT	5.8		FH	1.6
LB	4.4		PP	14.5
JW	12.5		JF	9.27
			BD	22.1
Children:				
Rh	1.2	1.3	Gu	7.85
De	2.1	10.1	Ge	5.4
Me	6.1	2.1	Da	6.4
Ho	4.85	9.0	Kr	4.8
Dn	8.2	3.45	Mc	3.87
Ro	1.86	6.0	Kr	2.3
			Ma	5.9

urine were too low to be either accurate or reproducible. Attempted modifications of this technique to include preliminary treatment for removal of interfering substances (such as histidine and cystine) also failed to give consistently reproducible results.

Harper, Kinsell and Barton have studied plasma and urine levels of methionine after intravenous injection,[6] using a microbiologic assay which measured the 1- isomer. Their data show a pre-injection level of 0.1 mg. per hour, and they found that for 3 hours after intravenous injection, 1.6 to 6.5 mg. of an injected 750 mg. were recovered from the urine. Hui Lan Yeh, *et al.*[8] studied the amino acid excretion in the urine of a patient suffering from congenital cystinuria. Using a microbiologic

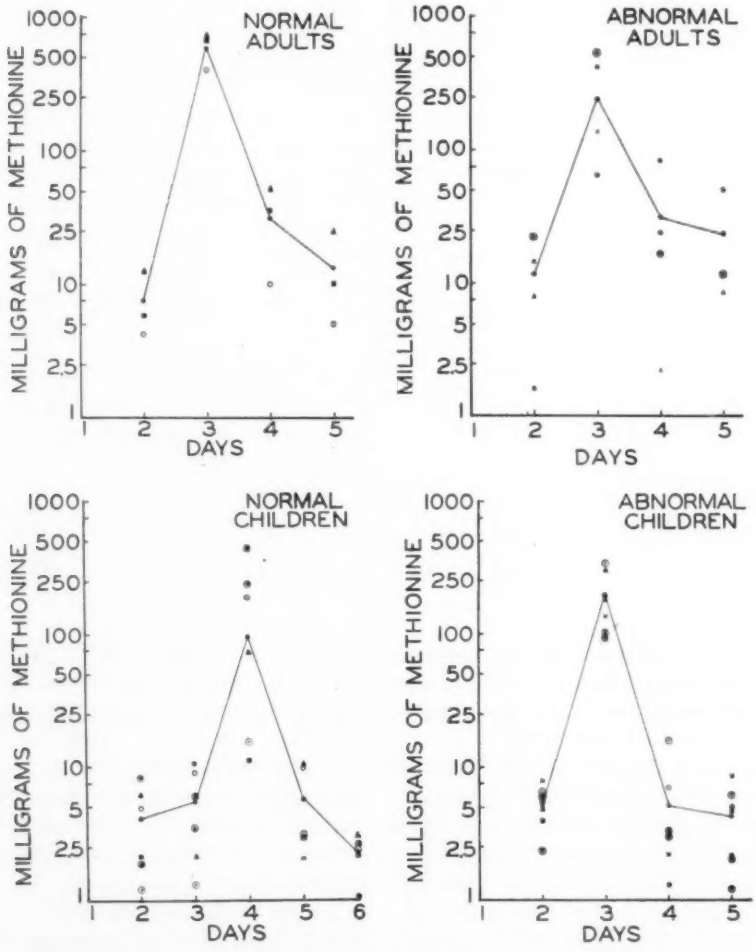

FIG. 1.—Excretion of supplementary dietary dl-methionine by normals and by patients with liver disease. *A*, The excretions are entered in the graphs at the end of each 24-hour period. Thus, the values shown at 2 days represent the pre-ingestion excretion. Methionine was administered at the beginning of the second day. The group of normal children received methionine at the beginning of the third day. *B*, Abscissæ (milligrams of methionine found) are plotted on a logarithmic scale. *C*, Solid circles connected by lines are averages of values found. Each other symbol represents an individual determination on a patient. *D*, In the chart of "Normal Children" the lowest point represented by a black square corresponding to the 4th day is not included in the average. An unknown, probably major, portion of this 24-hour specimen of urine was lost.

method which measured both d- and l-forms of methionine, they found values of 3 −25.5 mg. per 24 hours (mean of 11.03). Subsequently Dunn[5] has studied the urinary excretion of 12 amino acids by normal subjects using the same method; he found values for methionine averaging 2.7 mg. per 24 hours.

and has reached an average of only 10% of the supplementary dose of methionine given. Thus, it may be assumed that in hepatic conditions methionine may be utilized apparently to the same extent as in normal persons. It seems that the liver damage must be extensive before significant alteration of urinary excretion of exo-

TABLE 3.—FREE AND COMBINED METHIONINE FOUND IN FRACTIONAL SPECIMENS OF URINE COLLECTED IN A 24-HOUR PERIOD FOLLOWING INGESTION OF 3 GM. BY A FASTING NORMAL ADULT SUBJECT

	Fasting	Interval				
	8:00 A.M.	1:20 P.M.	5:30 P.M.	11:30 P.M.	8:00 A.M.	Total
Volume, in ml.	330	345	215	500	595	1655
After urease treatment, dl-methionine as micrograms/ml.	9 8	967	405	111	17	
Mg. dl-methionine	3 2	334	87	56	10	487
After HCl hydrolysis dl-methionine as micrograms/ml.	15 3	1045	980	192	52 5	
Mg. dl-methionine	5 0	361	210	96	31 2	698
Ratio, combined/free	1 55	1 08	2 41	1 63	3 12	1 43

The results of this study confirm the latter findings, all of which are based on a microbiologic assay. Consistent recovery of known amounts of methionine added at various steps in the assay procedure confirm the reliability of this method. The fact that smaller amounts of methionine can be detected by, and are found by this method furnishes evidence that the microbiologic assay is more specific than chemical methods. The high values found[1,7] on chemical determination for normal urinary excretion of methionine (250 to 500 mg. per day) in contrast to the much lower corresponding figures (2.5 to 15 mg. per day) of the microbiologically determined urinary methionine, are due probably to error inherent to the chemical methods in question, when applied in biological fluids.

In one group of patients suffering from hepatic disease the urinary excretion of methionine has shown no significant difference from values found in normal persons. Ingestion of additional methionine in one large dose (3 gm. to children, 5 gm. to adults) has produced both in normal persons and in patients with hepatitis or cirrhosis, a sharp rise in the urinary figures of methionine. However, the total amount excreted was found to be relatively low, genous (or endogenous) methionine can be found.[9]

Slight prolongation of high methionine levels in the urines of patients suffering from hepatic disease would appear to be the only deviation from excretion by normals. This difference manifested itself only in the group of adults with their more severe hepatic conditions and was not apparent in the group of children suffering from mild hepatitis. Interpretation of this finding must include evaluation of the use of dl-methionine. Although the occurrence of d-amino acids in proteins has been reported,[12] their significance in human metabolism has not been established. Since Lactobacillus fermentii-36 measures both enantiomorphs, the isomeric type of the methionine found in these studies remains to be established. It is not unlikely that the increased post-ingestion values represent renal spillover from a transient hyper-d-methioninemia produced by administration of dl-methionine in amounts greater than physiologic needs. This explanation is supported by finding low basal excretion levels, suggesting that methionine is ordinarily almost completely metabolized, even in patients suffering from hepatic disease.

In severe cirrhosis, the absorption of methionine (solution of crystalline dl-

methionine) from the small intestine is slightly impaired, as shown by Machella in this laboratory.[11] It is questionable whether this slight delay in absorption represents a satisfactory explanation for the prolongation of high methionine levels in the urine, extending over several days after the ingestion of one dose.

Summary. The urinary excretion of methionine has been studied by means of the microbiologic assay method (Lactobacillus fermentii-36) in groups of 3 normal adults, and 6 children as well as of patients of similar age distribution (4 adults and 7 children) suffering from mild or moderately severe hepatic disease (hepatitis, cirrhosis). One large supplementary dose of dl-methionine produced after ingestion a considerable but transient rise in the urinary excretion of methionine in all groups. However, the relative total amount excreted remained low throughout (approximately 10% of the dose given). Slight prolongation of the high methionine levels in the urine of adults suffering from moderately severe hepatic disease constituted the only difference from excretion by normals.

It is concluded that methionine is well utilized by patients with hepatic disease of mild or moderately severe nature.

Our gratitude is due to John G. Reinhold, Ph.D., for helpful advice and to Miss Jane Joraleman for technical assistance.

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URINARY EXCRETION OF NIACIN AND RIBOFLAVIN IN PATIENTS WITH ACUTE INFECTIONS AND VARIOUS CHRONIC DISEASES*

By David Cayer, M.D.
ASSISTANT PROFESSOR OF MEDICINE
AND
Sophia Cody, M.D.
RESEARCH ASSISTANT
WINSTON-SALEM, NORTH CAROLINA

(From the Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, and the North Carolina Baptist Hospital)

The association of famine with disease has been recognized since the beginning of recorded history. Recently a definite relationship between diet—chiefly the intake of vitamins and proteins—and susceptibility to infection has been shown in numerous animal experiments. This relationship does not appear to be the same for bacteria and viruses; however, it has also been shown that poorly nourished animals are actually more resistant to certain viral infections, particularly poliomyelitis and vaccinia, than are well nourished animals. This difference in susceptibility to bacterial and viral infections may be dependent upon the requirements of the host or invading organism for certain metabolic essentials, as well as upon the localization (intracellular or extracellular) of the parasite. At any rate, it is evident that vitamin deficiencies do not invariably lead to lowered resistance to all infectious diseases, although it is thought that a well balanced diet is important in maintaining a defense against infection and in speeding the recovery from bacterial infections.

The Prophylactic Administration of Vitamins. Vitamins are often administered to patients on the hypothesis that they may increase their resistance to bacterial infections. This practice has resulted in the indiscriminate use of excessive quantities of expensive vitamin preparations where little, if any, indication for such treatment is present. The disappointing results of such therapy in the prophylaxis of upper respiratory infections and in the treatment of such conditions as infectious arthritis are familiar to all practitioners.

The Supportive Administration of Vitamins. It is usually assumed that during an acute infection or a chronic illness, particularly where fever and disturbances of metabolism are present, the requirements for vitamin substances are increased. Marked evidences of protein catabolism have been demonstrated in Rocky Mountain spotted fever, for example, and it has been shown that this disease can be more successfully managed by the forced feeding of a high-protein diet.[4] The marked increase in protein and carbohydrate metabolism in such illnesses suggests that the demands for the B-complex vitamins may also be increased. The use of vitamins as supportive therapy in infections and in chronic diseases is still largely empiric, however, particularly in human beings. An estimation of the vitamin needs of ill patients would depend upon the preceding diet and degree of tissue storage, as well as upon the intake, absorption, utilization, and actual requirements during the illness. Such data are rarely available. The importance of niacin and riboflavin in the metabolism of carbohydrates and amino acids would seem to indicate that these vitamins have vital rôles in promoting recovery from injury and infection.

The Recognition of Vitamin Deficiencies. It is generally agreed that for each case of a severe deficiency state recognized by clinical means alone, many

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instances of mild deficiencies probably exist. Attempts at evaluating such states of nutrition are difficult, and, as a result, concepts of deficiency diseases have been somewhat confused: the clinicians state that frank evidences of dietary deficiencies are growing less common, while the nutritionists continue to maintain that routine surveys reveal marked evidences of inadequate diets. The recognition of mild vitamin deficiencies by clinical examination alone is often difficult, if not impossible. A gradual depletion of body reservoirs, with subsequent lowering of the concentration of vitamin substances in the blood and urine, precedes functional disturbances, microscopic tissue changes, and gross anatomic changes.

The development of laboratory procedures for the purpose of measuring vitamin substances in the urine and estimating body stores by the use of a test dose of vitamins appears to offer a means of determining actual vitamin needs. Previous studies have shown that the results of these laboratory tests correlate well with the physical evidences of deficiency states.[10] In an effort to obtain more accurate information concerning the nutritional states and requirements of patients with acute and chronic illnesses, the present study was undertaken to determine the amounts of niacin and riboflavin excreted in the urine of hospitalized patients who had acute infections and various chronic diseases.

Selection of Patients and Method of Study. Three groups of hospitalized patients were studied. Group 1 consisted of 13 patients with acute infections such as streptococcal pharyngitis, acute rheumatic fever, Rocky Mountain spotted fever, pyelitis, and pneumonia. Convalescence in these patients was usually noted by the end of the second or third week. Group 2 consisted of 68 patients with prolonged or chronic illnesses, such as heart disease, peptic ulcer, syphilis, chronic pulmonary disease, and chronic pyogenic infections. Group 3, which served as a control, was made up of 22 hospitalized patients who had no evidence of organic disease.

None of the patients studied had received any previous vitamin therapy. During the period of study all patients were given a bland diet containing 1800 to 2000 calories daily, divided as follows: protein, 60 to 70 gm.; fat, 80 gm.; carbohydrate, 100 gm. This diet was estimated to contain 2.9 mg. of riboflavin and 12.7 mg. of niacin.

Determinations of niacin and riboflavin were made on urine collected in dark-brown glass bottles for protection against light. Glacial acetic acid and toluol were used as preservatives. Three consecutive 24-hour collections of urine were made, the amounts of niacin and riboflavin contained in each were determined, and the results of the determinations were averaged. On the morning of the fourth day, 5 mg. of riboflavin and 50 mg. of niacinamide were given orally with breakfast, after which a 3-hour urine collection was made.

The niacin assays were done by the method of Perlzweig[5] for the determination of urinary F_2 (N-methylnicotinamide). The riboflavin levels were determined by the direct fluorometric method of Ferrebee, omitting the absorption and elution steps.[3]

Results. The urinary levels of niacin and riboflavin for the various groups are seen in Tables 1 and 2. Before the administration of a test dose of niacinamide, little difference in the amount of F_2 excreted was noted between Group 3 (controls) and the other groups studied. The slightly higher urinary levels of F_2 in the groups with acute infections and cancer were not found to be statistically significant.

Following the administration of 50 mg. of niacinamide, the amount of F_2 excreted by the patients with acute and chronic infections was higher than that excreted by the controls. The difference was subjected to statistical evaluation by the "t" test and was found to be significant.*

The urinary excretion of riboflavin did not vary significantly in any group except in the patients with peptic ulcer. Here, as

* Statistical analysis was done by Dr. C. N. Herndon of the Division of Medical Genetics.

might be expected, where the previous diet had, in most instances, been rich in sources of riboflavin (milk and cereal), the levels of urinary riboflavin before and after the test dose were 20 to 25% higher than those in the control group.[2]

ies does not support such a belief and the facts do not justify such a conclusion.

LIBERATION OF VITAMINS FROM STORAGE DURING ILLNESS AND STARVATION. It is possible that increased amounts of these vitamin substances are liberated

TABLE 1.—URINARY EXCRETION OF NIACIN

	No. cases	Before test dose: Range (mg.)	Before test dose: Mean (mg.)	After test dose: Range (mg.)	After test dose: Mean (mg.)
Group 1:					
Acute infections	13	3.9–11.7	8.5	4.0–14.2	7.1*
Group 2:					
Chronic diseases	83				
Cancer	15	4.6–13.9	8.4	1.1– 8.2	3.7
Pyogenic infection	4	5.6–10.5	9.0	3.7–12.1	6.8*
Syphilis	4	1.9–17.6	7.0	1.6– 6.9	3.6
Lung disease	3	6.3– 7.4	6.9	3.1– 5.8	3.7
Heart disease	17	2.9–14.8	6.9	0.6–10.1	3.8
Peptic ulcer	25	3.2–15.5	6.7	1.1–15.2	4.6
Miscellaneous	15	1.2–11.9	4.2	0.5–11.0	4.0
Group 3:					
Controls	22	2.5–12.2	7.2	1.0– 6.7	2.9

* Statistically significant.

TABLE 2.—URINARY EXCRETION OF RIBOFLAVIN

	No. cases	Before test dose: Range (γ)	Before test dose: Mean (γ)	After test dose: Range (γ)	After test dose: Mean (γ)
Group 1:					
Acute infections	13	674–6393	1682	440–3128	2194
Group 2:					
Chronic diseases	83				
Cancer	15	475–2512	1246	532–4268	1708
Pyogenic infection	4	994–2573	1645	908–2550	1597
Syphilis	4	944–2009	1490	464–2288	1761
Lung disease	3	1212–2260	1738	816–2380	1851
Heart disease	17	576–4595	1621	1302–3488	2026
Peptic ulcer	25	622–6311	2003	520–4830	2421
Miscellaneous	15	712–2640	1440	424–3720	2172
Group 3:					
Controls	22	640–4224	1482	232–6771	2025

Discussion. In evaluating the results of the present study certain apparent discrepancies are noted. The quantity of niacin and riboflavin excreted by ill patients, contrary to what might be anticipated, is as great or greater than that excreted by the controls. While this finding might be interpreted to indicate that the nutritional state of the former patients was better than that of the latter, the evaluation of their previous dietary histor-

from storage incidentally, or perhaps as a protective mechanism, by fever, an increased rate of metabolism, and tissue destruction. It would appear that until the body reserves are depleted, excretion of these substances at the onset of an illness may actually be greater than normal. In healthy subjects on a voluntary fast relatively normal urinary levels of niacin have been found.[7] In addition, the work of Meehclson and Erickson with normal

subjects has shown that urinary F_2 (N-methylnicotinamide) levels, even after periods of restricted diet, are maintained for a period as long as 32 days, with only a slight decrease. The niacin and riboflavin excreted by such persons are apparently derived from the tissues which contribute the urinary nitrogen.

The normal or elevated urinary levels of F_2 and riboflavin found in Groups 1 and 2 suggest the following conclusions:

That in most instances the amounts of niacin and riboflavin stored in the tissues appear to be adequate to supply the requirements of patients studied during fairly long periods of illness before gross anatomic tissue changes appear; this assumption would account for the relative infrequency of frank clinical deficiencies even during prolonged illness.

That during illness, particularly when fever and weight loss are present, normal or increased amounts of riboflavin and niacin may be excreted until the body stores become depleted. Thus it can be understood why frank deficiencies, though they are uncommon even during relatively prolonged illness, are more likely to appear in patients with chronic diseases, since their stores are being more rapidly depleted.

The Rôle of the Liver. Another possible factor in the normal to increased excretion of F_2 by ill patients may be the inability to metabolize N-methylnicotinamide in the presence of liver damage. The liver is apparently the chief, if not the sole, organ responsible for the methylation of niacinamide.[6] N-methylnicotinamide is further broken down by the liver to unknown products. Experimental carbon tetrachloride poisoning in rats diminishes the capacity of the animals to destroy N-methylnicotinamide.[8] A similar effect has been noted in human patients with liver disease[1] as well as in apparently normal females during pregnancy.[9]

While the urinary level of F_2 aids in the evaluation of an individual's nutritional state, it would appear that it also serves as a measure of his ability to methylate and metabolize niacinamide. It is possible that in the presence of infection, particularly where liver function is disturbed, there is interference with the latter process. The highest urinary F_2 levels were found in a patient with Rocky Mountain spotted fever who had evidence of severe disturbance of liver function.

Application to Therapy. It is difficult to say whether or not the increased urinary excretion of niacin demonstrated in patients with acute and chronic infections indicates a need for increased vitamin intake. Where recovery occurs before the body stores are depleted, a satisfactory adjustment is apparently made during convalescence. Since the retention of some of the members of the B complex may be dependent upon the storage of protein, it might be advantageous to supplement the niacin and riboflavin intake following illness. In most instances, the amounts employed (5 to 15 mg. of riboflavin and 50 to 150 mg. of niacin) appear to be considerably larger than necessary. Further study of these aspects of the problem is in progress.

Summary. Assays of the urinary excretion of niacin and riboflavin in hospitalized patients having acute and chronic illnesses differ little from those in a control group of patients, without organic disease. Following the administration of a test dose of these vitamins, the amount of F_2 excreted by the patients with acute and chronic infections is significantly increased. The normal or increased levels in such patients are probably dependent upon liberation of niacin from storage as a result of fever, increased metabolism, and tissue destruction. The increased excretion of F_2 may also be related to disturbance of the liver function and to a diminution of the liver's capacity to metabolize N-methylnicotinamide.

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GANGRENE OF THE EXTREMITIES OF VENOUS ORIGIN

REPORT OF A CASE

By HENRY HAIMOVICI, M.D.
ADJUNCT ATTENDING SURGEON, MONTEFIORE HOSPITAL
NEW YORK, NEW YORK
AND
GUSTAVE SUFFNESS, M.D.
ASSISTANT ATTENDING IN MEDICINE, ALEXIAN BROTHERS HOSPITAL
ELIZABETH, NEW JERSEY

THE common clinical course of the occlusion of the main vein of an extremity is well known. The absence of anoxemia of the tissues in thrombophlebitis or phlebothrombosis is explained by the ease with which the collateral circulation is reestablished, due to the abundant venous pathways.

When, however, in addition to the main vein, all the tributaries are also occluded, marked anoxemia of the tissues leading to gangrene may ensue from the sole obstruction of the whole venous tree.

Gangrene, as a result of thrombophlebitis without arterial occlusion is, however, of rare occurrence. Only a small number of such cases have been reported.[1] In the American literature there is only 1 paper; it reports 3 cases.[8] The possibility of gangrene due to venous obstruction alone is therefore not widely known. Because of the rareness of the condition we wish to report the following case.

Case Report. M. L., 62 years old, white male, was admitted on May 26, 1946, to the Alexian Brothers Hospital, Elizabeth, N. J., for neoplasm of the bladder. The patient had hematuria for about 2 months prior to his admission. Except for a right inguinal hernia and some degree of chronic bronchitis, the patient's general condition was good. At the time of his admission an electrocardiogram was normal and the blood pressure was 150/70. On May 27, 1946, a midline suprapubic cystotomy was performed (by Dr. Irving Lerman). The tumor was resected and 5 radon seeds of 1.5 m.c. were inserted. The patient was ambulatory 48 hours after the operation.

On the 4th postoperative day, the patient developed hiccoughs, for which he received carbon dioxide. Three days later congestion of the right lower lobe was diagnosed and confirmed by a Roentgen ray plate the following day. The patient was running a low fever (101° F.) but there was no tenderness along the lower extremities. He was started on 20,000 units penicillin every 3 hours. On June 7 the left calf appeared infiltrated and tense. The diagnosis of thrombophlebitis became apparent. The patient was then put on dicumarol with 100 mg. as an initial dose, papaverine gr. ½ was given subcutaneously every 4 hours and the leg was elevated. During the following 3 days the calf was distinctly tense and the foot was warm. The patient's general condition was good.

On June 11 (15th postoperative day) at 5 P.M. the patient experienced a sudden pain in the left leg and foot, more marked in the latter. Upon examination, the entire left lower extremity, from the hip down, was swollen and warm, with the exception of the foot and lower third of the leg, which were cold and mottled. The circumference at the calf was 38.5 cm. as against 32 cm. on the opposite side. The dorsalis pedis and the posterior tibial arteries were felt and of good volume. Oscillometric readings were:

	Ankle	Calf
Left	2.5	3.5
Right	2.25	3.5

A paravertebral block with 1% novocaine was performed at L_1, L_2, L_3, and L_4, with good immediate results. The severe pain, present before the block, subsided and the patient remained comfortable during the next 24 hours. Nevertheless, the following day, all the toes appeared much more cyanotic than on the previous examination. Another paravertebral block with novocaine was performed with immediate good results. Despite that, within the next 24 hours, the first and fifth toes appeared completely

black, while the second, third, and fourth ones were black only distally. The areas of gangrene, involving the toes, were more marked on the plantar region. In addition, the ball of the foot and a circumscribed area near the heel also appeared gangrenous (Fig. 1). Two days later, another block was performed with the same apparent favorable response. The patient was much more comfortable. A line of demarcation became evident 4 to 5 days after the onset of gangrene.

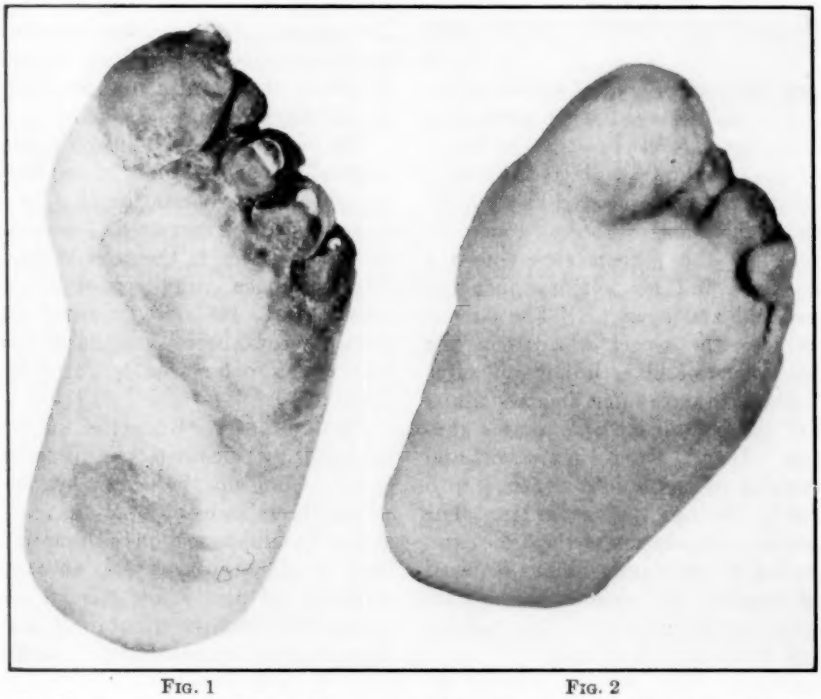

FIG. 1 FIG. 2

FIG. 1.—Plantar region of left foot. Gangrene involving the 5 toes. Additional superficial necrotic lesions are present on the ball of the foot and near the heel.

FIG. 2.—The healed stumps of the 5 toes after spontaneous amputation, 11 months after the onset of gangrene.

On June 19, the circumference at the calf was 33.5 cm. (5 cm. less than 8 days previously). The edema of the entire left lower extremity was subsiding. The areas of gangrene on the plantar region (ball and heel of the foot) showed some fading, while those of the toes remained unchanged.

On July 1 (3 weeks after the onset of the acute ischemic episode) the patient was allowed to be out of bed, and 6 days later he was discharged from the hospital. His general condition was good. The edema of the lower extremity had completely subsided, and the gangrene of the left 5 toes was dry and well demarcated. No active surgery was contemplated, for it was assumed that spontaneous amputation would eventually occur. Indeed, the lesions of the ball and heel remained superficial and separated in a few months. As to the toes, after the demarcation between the normal and dead tissues began, the separation of the gangrenous distal phalanges took several months (from October 9, 1946, to February 3, 1947). The stumps (Fig. 2) of the different toes were completely healed in May, 1947, 11 months after the onset of gangrene.

While at home, on September 2, 1946, 2 months after his discharge from the hospital, the patient developed a typical ilio-femoral thrombophlebitis of the right lower extremity. The entire limb was markedly swollen, hot and painful. The patient was running a fever of 101.5° F. The next day a right paravertebral block was performed and re-

peated 24 hours later. Within 24 hours after the last block the temperature became normal and the pain subsided. The edema of the extremity subsided within a week. Unlike the thrombophlebitis of the left lower extremity, that of the right side was not accompanied by ischemic phenomena and followed a normal course.

When last seen in June, 1947, all pulses were present in both feet, and the oscillometric readings were normal. It is to be emphasized that throughout the entire course of the disease the foot arteries remained patent and the oscillometric readings were normal.

Discussion. The clinical course of the case we have observed went through 4 distinct phases. *First phase:* at the inception, during the first few days, the thrombophlebitic process appeared as a typical phlegmasia alba dolens. *Second phase:* on the 5th day the patient experienced a sudden pain and his left leg and foot became cold and cyanotic. The clinical picture took the aspect of a phlegmasia cærulea dolens or blue thrombophlebitis. *Third phase:* 3 days later, the toes and 2 areas of the plantar surface became gangrenous. *Fourth phase:* regression and demarcation of the lesions began 4 to 5 days after the onset of gangrene. The spontaneous amputation of the 5 toes and the healing of the plantar lesions required several months. At present there are no post-phlebitic sequelæ and the walking capacity of the limb is normal.

Diagnosis. The first 3 stages are typical of the course of a thrombophlebitis resulting in gangrene. At the second and third phases, however, it may be difficult to differentiate between an acute venous and arterial occlusion. Indeed, a syndrome characterized by a sudden onset of pain, cyanosis and coldness of an extremity, as seen in the second phase, may correspond either to an acute blue thrombophlebitis or to an acute arterial occlusion. In our case the presence of edema and the patency of the foot arteries were in favor of the former diagnosis. In certain instances arterial spasm may accompany the onset of the venous occlusion, in which case the latter is easily mistaken for an arterial embolism.[2,5] The use of intravenous papaverine or a paravertebral block may be of great help in the differentiation of these 2 conditions. In some cases with prolonged spasm the true nature of the occlusive vascular syndrome has been established only during surgical exploration of the main vessels.[5,6,7]

At the third phase, the examination of the pulses and the oscillometric findings are decisive in establishing the diagnosis. The patency of all the arteries and normal oscillometric readings should rule out the diagnosis of gangrene due to arterial or mixed arteriovenous occlusion.

The prognosis of post-phlebitic gangrene is usually poor. In most of the instances recorded in the literature, leg or thigh amputation was required. A favorable outcome, as seen in the present case, is of rare occurrence. Another such instance in which there was only minimal loss of tissues (spontaneous amputation of the toes) was also reported by Fontaine and Forster.[3]

Pathogenesis. Gangrene of venous origin is a distinct clinico-pathologic entity. Clinically the venous syndrome is a blue thrombophlebitis, resulting in gangrene. Careful dissection of the amputated limb, or at autopsy, reveals an extensive occlusion of the whole venous system (main vein and its tributaries) and the absence of any organic lesion of the arterial tree. Experimentally[4] it has been shown that this type of gangrene could be reproduced in the animal, provided the blockage of the venous system is complete.

Since in some cases there is evidence of arterial spasm accompanying the thrombophlebitic process, the question arises as to how much it is responsible for the gangrene. From the available data, arterial spasm appears to play rather a minor rôle if any, in its causation. Our present case supports this view, since at no time during the course of the disease was there any evidence of spasm.

As to the mechanism, the clinico-pathologic and experimental data seem to

indicate that, although the arterial blood could reach the tissues, its return is prevented by the blockage of the venous system, leading thus to stasis and anoxia of the tissues, resulting in gangrene.

TREATMENT. From a therapeutic standpoint, it is obvious that the management of this condition will vary according to the phase. In the beginning, at the phlegmasia cærulea dolens stage, the use of papaverine and/or paravertebral block of the sympathetic ganglia and anticoagulants should prove effective against the angiospasm and further extension of the venous thrombosis.

When gangrene sets in, and the diagnosis of its venous origin is certain, it is important to remember that the necrosis may remain superficial. Delaying amputation seems therefore indicated, unless signs of toxemia are present.

Summary. 1. A case of gangrene of the lower extremity due to venous occlusion is reported.

2. The clinico-pathologic characteristics of this type of gangrene, namely, extensive thrombosis involving all the veins and patency of the arterial system, are described.

3. Pertinent therapeutic indications are stressed.

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IN VIVO STAINING AND RETARDATION OF TUMORS IN MICE BY ACRIDINE COMPOUNDS*

By Margaret Reed Lewis,
MEMBER OF THE WISTAR INSTITUTE
AND
Philip P. Goland, M.D.†
PHILADELPHIA, PENNSYLVANIA

(From the Wistar Institute of Anatomy and Biology)

In previous studies[14] (1946) it was shown that acridine dyestuffs of Colour Index No. 788 added to the diet of tumor bearing mice stained sarcomata and spontaneous carcinomata and retarded their growth. In addition to 36 commercial dyestuffs studied at that time, 34 acridine compounds synthesized as antimalarials‡ were tested on tumor bearing mice, and, five were found that stained and retarded tumors in mice.

Acridine compounds have been extensively synthesized and studied as dyestuffs, antibacterial and antimalarial agents. Reviews on the latter two aspects have been furnished by Albert, Rubbo, Goldacre, Davey and Stone[1] (1945); Martin[17] (1944); and Wiselogle[22] (1947).

Through the generous collaboration of Dyestuff Manufacturers and of Laboratories§ which synthesized compounds for studies on Malaria, we were able to obtain samples, together with information regarding their structural formulæ, solubility, and when known, their toxicity, for our studies on the structural nature of compounds that stain and retard tumors in mice. The results of these investigations which include 296 acridine antimalarials and 50 dyestuffs are reported in the present communication.

Material and Method. The mice used were 40 to 50 days old inbred Bagg albino (our own) and C57 black strains (our own), weighing 18 to 20 gm. bearing transplanted sarcomata, (BA, No. 1 and C57, No. 241, originally induced by means of 1:2:5:6-dibenzanthracene), and mice of the dilute brown, J. W. Thompson's C3H, and inbred albino strains bearing spontaneous mammary gland carcinomata.¶ In some experiments mice of the Andervont C3H strain ingrafted with a carcinoma that arose spontaneously in a mouse of this strain were also used.

The acridine antimalarial compounds were ground together until pulverized with Purina fox chow meal in amounts equivalent to 0.1 and 0.15%, the dyestuffs to 0.4%, of the food weight. These relatively large amounts were administered in order to establish their maximum staining and retarding action on tumors and their toxicity to mice. Diets containing 25 different compounds were prepared and placed in amber glass jars with tight screw tops. Thirty mice of an inbred strain were then implanted subcutaneously with a measured small graft (5 x 2 x 1 mm.) of a 100% transplantable

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† Frances Clark Fellow in Neurosurgery and Fellow in Harrison Department of Research Surgery, University of Pennsylvania.

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¶ Presented to us from the Research Department of the Lankenau Hospital, Philadelphia, Pa.

sarcoma native in the strain used, placed in individual containers and given 3 to 5 gm. of food each (*i. e.* slightly more than the amount utilized by a normal mouse); 24 of them receiving treated and 5 untreated chow. Records of food intake were not made.

Samples of urine were examined, and the mice were sacrificed about 14 days later by which time those receiving untreated food were ill or moribund with large tumors. The treated mice were sacrificed at this time because sufficient quantities of only a few of the compounds were available for longer studies.

As each mouse was sacrificed records were made of its condition and of the size and color of its organs and tumor. If the mouse had grown well (mice were not weighed), was in good condition, and its tumor was large and uncolored, the test was not usually repeated. Tests on mice that became ill or did not grow were repeated using less compound. Compounds that stained and retarded sarcoma were tested on mice bearing spontaneous mammary gland adeno-carcinomata.

The acridine compounds tested numbered 331; 27 had no amino group, 269 had one amino group and 35 had two amino groups situated upon the acridine structure. Of the mono-amino and di-amino compounds 204 stained tumor tissue. The majority of the compounds that stained tumor tissue brought about some retardation of its growth.

Results. *Acridine Dyestuffs.* The results of our studies on acridine dyestuffs were the same as those previously reported, namely, dyestuffs of Colour Index No. 788 stained and retarded tumors in mice; those of other Colour Index numbers failed to stain tumors. Many dyestuffs were toxic in concentrations equivalent to 0.4%, the amount used in our earlier studies with commercial dyes, but not in 0.2%. Samples of acridine orange were purified by the American Cyanamid Company (Calco) and E.I. duPont de Nemours & Company to see whether the toxicity of commercial dyes might be due to the presence of impurities.

Acridine Antimalarials. Oral administration of 49 of them, in amounts equivalent to or less than 0.15% dry weight of their food, resulted in stained tumors that were smaller than those in untreated mice of the same series; 16 of them retarded the growth of tumors to such an extent that they were 1/20 to 1/40 the size of those in the controls (Table 2).

The majority of the 127 compounds that failed to stain tumor tissue, also failed to retard its growth and only a few resulted in unstained tumors that were less than 1/10 the size of those in untreated mice of the same series (Table 3).

A few of the compounds brought about marked retardation of tumor growth even when administered in low concentration, 0.05% to 0.03%. These were WIC-37, WIN-340, and WIN-501 (see Tables 2 and 3). Although these compounds were toxic when fed in amounts equivalent to 0.1%, when used in lower concentrations the treated mice remained healthy.

Many of the compounds were excreted in bile and urine. Colored urine did not necessarily mean that the animal's tumor would be colored; on the other hand, tumors were not stained in animals whose urine was normal in color.

As mentioned in previous studies[14] (1946) staining of tumor tissue by compounds given by mouth results in diffuse coloration throughout the tumor tissue and is not located in granules in tumor cells, in cells of necrotic areas or in cells of adjacent tissues.

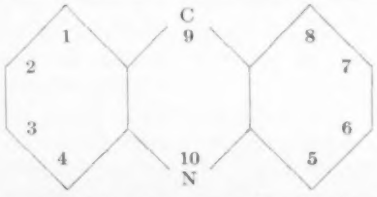
The lymph nodes, thymus, testicle and intestinal walls of mice in which tumor tissue was stained were also diffusely stained a paler tint of the same color and in a few the medulla of the adrenal was diffusely stained. In addition to this diffuse staining, darker yellow granules were found in epithelial cells covering intestinal villi, in macrophages within villi, in cells of a few collecting tubules of the kidney, and in cells composing the kidney medulla. The liver was yellow in mice that received certain of the compounds.

Some tumors were pale yellow (WIC-27), some bright yellow (ERS-343), and some orange color (A-79). The intensity of the growth retarding action was not associated with the color brought about in the tumor.

TABLE 1. STAINING RESULTS WITH THE VARIOUS COMPOUNDS TESTDE

No amino group	No.	Stained	Unstained
9, 10-position substituents	16	0	16
2-methoxy-9, 10-position substituents	1	0	1
3-chloro-9, 10-position substituents	1	0	1
1,2,3,4-tetrahydro-9, 10-position substituents	1	0	1
2-methoxy-6-chloro-9-position substituents	6	0	6
2-methoxy-6-chloro-9, 10-position substituents	1	0	1
	26	0	26
Monoamino	No.	Stained	Unstained
9-amino	6	3	3
2-chloro-9-amino	1	1	0
3-chloro-9-amino	3	2	1
4-chloro-9-amino	2	0	2
2-methoxy-9-amino	2	1	1
4-methoxy-9-amino	1	1	0
2-methyl-9-amino	1	0	1
4-methyl-9-amino	3	1	2
Benzo(a)-9-amino	3	2	1
Benzo(c)-9-amino	3	2	1
Benzo(a)-6-chloro-9-amino	1	1	0
3-phenyl-imino-9, 10-position substituents	1	0	1
2-bromo-4-methyl-9-amino	1	1	0
2-chloro-4-methyl-9-amino	1	0	1
3-chloro-4-methyl-9-amino	1	0	1
3-chloro-5-methyl-9-amino	1	1	0
2-methoxy-4-chloro-9-amino	1	0	1
3-nitro-6,7-dimethoxy-9-amino	5	3	2
2-hydroxy-6-chloro-9-amino	1	0	1
2-methoxy-6-chloro-9-amino	212	158	54
2-methoxy-7-chloro-9-amino	2	1	1
2-methoxy-6-cyano-9-amino	5	4	1
2-methoxy-6-methyl-9-amino	1	1	0
1-methyl-4-methoxy-9-amino	1	0	1
3,6-dichloro-9-amino	1	1	0
3,6-dichloro-4-methyl-9-amino	1	1	0
4,6-dichloro-2-methoxy-9-amino	1	1	0
6-chloro-2,3-dimethoxy-9-amino	1	1	0
9-(para-amino)phenyl-3-amino	6	0	6
9-(para-amino)phenyl-7-methyl-3-amino	1	0	1
	270	187	83
Diamino	No.	Stained	Unstained
3,6-diamino	2	0	2
3,6-dimethylamino	16	16	0
3,6-diacetylamino	1	0	1
10-methyl-3,6-diamino	4	0	4
9-phenyl-3,6-dimethylamino	1	1	0
2,7-dimethyl-3,6-diamino	4	0	4
2,7-dibromo-3,6-diamino	1	0	1
2,7-dimethyl-9-phenyl-3,6-dialkylamino	5	0	5
2,7,9,trimethyl-3,6-diamino	1	0	1
	35	17	18
Total	331	204	127

The compounds tested were listed according to the German scheme of classification:



(The acridine structure is so numbered that the meso carbon atom is at position 9. However, according to the manner of counting around the ring, similar compounds came differently named; for instance, 2-methoxy-6-chloro-9-amino acridine, or 7-methoxy-3-chloro-9-amino acridine.)

The amount of these compounds available for our studies was too small, in most instances, to permit studies on survival time of normal and of tumor bearing mice so that studies on prolonged feeding were not attempted. A few records were made, however, on the size of tumors in mice treated longer than usual. These showed that D-91, D-92, D-94, WIC-1, WIC-22, WIN-146, A-66 and quinacrine could be fed to tumor bearing mice for over a month in amounts that markedly retarded

TABLE 2.—RESULTS WITH STAINED TUMORS AND ONE-TWENTIETH TO ONE-FORTIETH THE SIZE OF THOSE IN UNTREATED MICE

Compound	Source	Size of tumor (c.mm.)	No. of days
3-chloro-7-methoxy-9-[γ-(β′-diethylamino-ethylthio)propyl-amino]acridine, di HCl	Sterling-Winthrop WIN-340	100	14
9-(ω-n-piperidylamylamino)-3,4-benzoacridine, 2HCl	Parke, Davis WIC-37	120	14
9-(4-dimethylamino-cyclohexylamino)-7-methoxy-3-chloro-acridine, 2HCl	Merrell 192R-92	200	14
2-bromo-4-methyl-9-(δ-diethylamino-α-methyl butylamino) acridine, di HCl	Lilly ERS-343	240	14
3-chloro-9-[γ(ethyl-β-hydroxyethylamino-propylamino)]-7-methoxy-acridine, 2HCl	Parke, Davis WIC-12	256	15
2-methoxy-6-chloro-9-(1′-n-propylisopropylamino-4′-pentyl-amino) acridine, di HCl	Lilly A-79	300	16
2-methoxy-6-chloro-9-(1′-ethyl-n-propylamino-4′-pentylamino) acridine, di HCl	Lilly A-85	300	15
4-methyl-9-(δ-diethylamino-α-methyl butylamino) acridine, di HCl	Lilly ERS-340	340	15
2-methoxy-6-chloro-9-(4′-dimethylamino-anilino) acridine	Parke, Davis WIC-27	350	15
6-chloro-2-methoxy-9-(4-dimethylamino-butylamino) acridine, dihydrochloride	Sterling-Winthrop WIN-501	350	14
2-methoxy-6-chloro-9-(2′-diethylamino-4′-butylamino) acridine, di HCl	Lilly A-55	360	15
γ-(2-methoxy-6-chloro-9-acridylamino) propyl-β-di-ethylamino-ethyl sulfide, 2HCl	Parke, Davis WIC-42	360	14
2-methoxy-6-chloro-9-(1′-ethyl-isopropylamino)pentylamino acridine, di HCl	Lilly A-172	420	14
3-chloro-7-methoxy-9-γ-dibutylamino-propylamino-acridine, 2HCl	Parke, Davis WIC-25	432	15
2-methoxy-6-chloro-9-3-ethylamino-propylamino) acridine, HCl	Abbott D-91	480	15
Atabrine dihydrochloride	Marshall 225	455	14

Control mice died with tumors about 8000 to 11,000 c.mm. at 14 to 16 days.

TABLE 3.—RESULTS WITH UNSTAINED TUMORS AND ONE-TENTH TO ONE-FORTIETH THE SIZE OF THOSE IN UNTREATED MICE

Compound	Source	Size of tumor (c.mm.)	No. of days
6-chloro-2-methoxy-9-[γ-(γ-aminopropylamino)propylamino] acridine, citrate	Parke, Davis WIC-30	144	15
9-(4-dimethylamino-cyclohexylamino)-6,7-dimethoxy-3-nitro-acridine, 2HCl	Merrell 192R-190	248	14
6-chloro-2-methoxy-9-[γ-(4′-diethylamino-phenylamino)propyl-amino] acridine, 2HCl	Parke, Davis WIC-23	480	15
9-(δ-diethylamino-α-methyl) butylamino-acridine, di HCl	Lilly ERS-334	600	18
2-methoxy-6-chloro-9-cyano acridine	Lilly J-143	960	17
2-methoxy-6-chloro-9-γ-n(-pipecolino propylamino) acridine, di HCl	Lilly A-27	960	13
6-chloro-9-(α,α-dimethylethanol)-amino-2-methoxyacridine	Parke, Davis WIC-35	1050	14
9-α-hydroxy-γ-butenyl-9,10-dihydroacridine	Lilly AL-727	1071	17

their tumor growth.* In a number of instances mice bearing spontaneous mammary gland carcinoma remained in good condition with retarded tumors for more than 42 days when given food containing 0.1% brilliant orange 3R, Nyanza, C.I. No. 788.

Compounds that stained and retarded tumors resulted in atrophy of the thymus regardless of age, growth and general condition of the host. This action on the thymus resulted in loss of thymocytes. In every instance in which the tumor was stained the thymus was also stained. The factor that brought about atrophy of the thymus did not influence the behavior of other lymphoid tissue. The mesenteric lymph nodes, the pleural lymph nodes and those in the axilla and groin remained approximately normal in size even when stained by the compound.

Microscopic preparations showed the presence of many cells undergoing mitotic division in tumors in treated mice, even though the tumors in untreated mice increased in size more rapidly than those in treated ones. Tumors in untreated young adult mice about doubled in size every 36 to 48 hours; grafts grew into tumors 900 to 1000 c.mm. in 7 days and 8000 to 11,000 c.mm. in 15 days. Figures showing cells in prophase, metaphase and anaphase were abundant in preparations of retarded tumors; on the other hand few pycnotic nuclei occurred. From this it seems evident that multiplication took place slowly rather than that the drugs act as caryolytic poisons. In a few instances, where large enough quantities of compounds were available to prolong the treatment, the tumors continued to grow slowly and after many days (30 to 40) they became moderately large tumors.

Discussion. Studies on malignant growths have established a general relationship between the well being of the host and rate of growth of malignant tissue. Agents that lower the growth rate of the host retard the growth of malignant tissue. In our experiments it was found that consumption of food containing toxic substances as well as lowered consumption of food resulted in retardation of the growth of both host and tumor. On the other hand retardation of tumor was not necessarily accompanied by ill health of the host. Some healthy, well grown mice had large tumors, others small tumors, depending upon the compound they received.

The retarding action of certain compounds, for instance A-79 and D-91, was so consistent that they were used to test whether the addition of vitamins, nucleic acids, coenzymes, surface active agents, etc., would increase or decrease the retardation of tumor growth in mice fed these compounds. Our results indicate that addition of nucleotides and thiamine to the diet in some measure counteracts the action of acridine compounds, but they are not as conclusive as those of McIlwain[19] (1941) and Hegsted, McKibbin and Stare[11] (1945). McIlwain suggested that the nucleotides form complex salts with acriflavine components and that the dye might act in this manner to inactivate enzyme systems of which nucleotides are an essential part. He found that B. coli was inactivated by acriflavine, but could grow in the presence of acridine compounds with the addition of nucleotides which were not needed for growth of untreated bacteria.

* 2-methoxy-6-chloro-9-(3-methylamino-2-methyl-propylamino) acridine	Abbott	D-92
6-cyano-9-(3-ethylaminopropylamino)-2-methoxyacridine, hydrochloride	Abbott	D-94
9-(3′-diethylaminomethyl-4′-hydroxy-anilino)-6-chloro-2-methoxyacridine	Parke, Davis	WIC-1
9-(3′-n-butylethylamino-methyl-4′-hydroxyanilino)-2-methoxy-6-chloroacridine, 2HCl	Parke, Davis	WIC-22
9-aminoacridine hydrochloride	Sterling-Winthrop	WIN-146
2-methoxy-6-chloro-9-(4′-diisopropylaminobutylamino) acridine dihydrochloride	Lilly	A-66
9-(5′-allyl-3′-diethylaminomethyl-4′-hydroxyanilino)-6-chloro-2-methoxyacridine	Parke, Davis	WIC-2

Hegsted, McKibbin and Stare[11] found in studies on rats that the addition of atabrine to a thiamine deficient diet delays loss of weight, onset of symptoms, and death. Animals receiving low levels of thiamine along with atabrine showed greater weight gains than controls, so that atabrine seemed to have a "thiamine sparing" action.

Atrophy of the thymus occurs in mice due to age and to wasting diseases, but the loss of the cortical portion of the thymus in mice fed non-toxic, tumor retarding compounds was too constant to be attributed to the host's general condition. Van Herrswynghels[21] (1934), who studied the action of trypaflavin on the thymus and other lymphoid organs, claims that the manifestations presented by these tissues show that the thymocytes are different from lymphocytes. This view seems to be confirmed by our results. Atrophy of the thymus, however, cannot be attributed to the action of acridine compounds in particular since we found that it accompanied retarded tumor growth whether produced by acridine, rhodamine or oxazine dyes that stain tumor *in vivo*.

The influence of various compounds, particularly acriflavin, on mitosis has been studied by Dustin and Gregoire,[7] 1934; Mayer,[18] 1934; Dustin,[6] 1939; Brock, Druckrey and Herken,[4] 1939; and Andervont,[2] 1940. These investigators found that mitotic division could be interrupted in prophase and anaphase or stopped in metaphase by certain substances (trypaflavin, sodium cacodylate, colchicine and others) which they termed caryolytic poisons. The investigations of caryolytic poisons in general showed that tumor cells are not influenced by all substances that effect other tissues. Colchicine produces a greater accumulation of mitotic figures in the thymus, germinal lymph centers, glands of Lieberkuehn, testicles and tumor. Trypaflavin brought about only slight retardation of mitosis in tumor tissue while it caused intense reaction in the thymus. Dustin and Gregoire[7] found that tumor cells show great sensitivity to agents that excite mitosis (sodium cacodylate) and a great resistance to agents that retard mitosis (trypaflavin).

A few of the acridine compounds have been used as therapeutic agents in studies on cancer by Lewin[13] (1920); Marsh and Simpson[16] (1926); Blumenthal[3] (1931); and Lettré[12] (1941). Marsh and Simpson injected mice bearing spontaneous mammary gland carcinomata with acridine orange and with acriflavin intravenously and subcutaneously during an average period of 34 days. They found that the treatment had little therapeutic effect on tumor growth. Lettré reported that trypaflavin inhibited complete mitosis and that daily injections of 0.025 and 0.05 mg. into mice implanted with an "ascites tumor" delayed the development of the tumor and prolonged the life of treated animals. Other investigators have reported that trypaflavin does not effect mitosis of tumor cells. Ascites tumors, however, are formed by growth of malignant monocytes, not by fixed tissue cells (Farris and Yeakel,[8] 1944.)

Lewin[13] and also Blumenthal[3] employed trypaflavin and argoflavin intravenously in the therapy of human malignant tumors and reported that improvement followed for short periods of time. In our studies on mice it was found that while a number of acridine compounds had marked growth retarding action, none of them retarded an actively growing sarcoma or carcinoma sufficiently to have therapeutic value.

Structural Analysis. The acridine antimalarials were in solid, usually powder form, and the majority of them were hydrochloride or dihydrochloride salts. The solubility in water or some other solvent was known for many of the compounds. However, this failed to account for the power to stain or failure to stain tumor tissue which resulted from the administration of many of the 9-amino acridines.

Albert *et al.*[1] (1945) have demonstrated that highly basic acridines are almost completely ionized in solution to give cations, and that compounds which lack

amino groups have poor ionization and antibacterial activity. No data were available as to the ionization of the compounds tested for tumor staining. Therefore, we cannot determine whether adequate ionization is a prerequisite for staining, allowing other factors, such as side chain constitution and ring substituents, to become manifest. Indirect evidence, however, on the necessity of adequate ionization is the fact that all compounds lacking an amino group as a substituent of the ring failed to stain tumors. For diffuse staining of tumors, cationic ionization appears essential. When acidic groups were introduced into the side chains of the 9-amino compounds, the compounds did not stain.

In the group of the 2-methoxy-6-chloro-9-amino (substituted) acridines, from which the largest number of compounds were tested, it is difficult to decide from the structure of the 9-amino chain, the elements determining staining. Large additions to the molecule, as seen in 2-methoxy-6-chloro-9-[2-(2′-methoxy-6′-chloro acridylamino) ethylamino] acridine, interfered with coloration of tumor tissue. The majority of the hydroxy derivatives of 9-aminoacridine in which the hydroxyl groups were situated off of the alkyl-amino-alkyl chain did not stain tumor. Acranil, 3-chloro-7-methoxy-9-(2-hydroxy-3-diethylamino-propylamino) acridine, dihydrochloride, and Neo-acranil, 3-chloro-7-methoxy-9-(2-hydroxyethyl-amino-ethylamino) acridine, dihydrochloride, did give staining of tumors. Goetchius and Lawrence[9] (1944) found these two compounds less effective as bactericidal and bacteriostatic agents than other amino-acridines. It was found by Browning and Cohen[5] (1921) and Linnell and Stuckey[15] (1940) that the hydroxy derivatives of acridine were inactive against bacteria. Only two compounds, in which the hydroxy-groups were substituents of the acridine structure, namely 6-chloro-9-amino-2-acridol, hydrochloride, and 2-hydroxy-6-chloro-9-(1′-methyl-4′-diethylaminobutylamino) acridine, dihydrochloride, were available for tests on tumors and they failed to stain tumors.

Certain substituted 9-amino acridines such as 9-(4-diethylamino-1-methyl butylamino) acridine which did not stain tumors or become concentrated in tissues did so if a 6-chloro, cyano, nitro or methyl group was present. Concentrations in tissues dependent upon the 6-chloro substituent are described in the analyses of Taggart[20] (1946) who found that 2-methoxy-6-chloro-9-(3-ethyl-amino-propylamino)acridine became concentrated to a greater extent in tissues than 6 other compounds analyzed by him. If retardation of tumor growth as well as staining are considered, it became evident that the majority of the 9-amino acridine compounds that stain tumor as well as retard it show dialkyl-amino-alkyl-amino chains in the 9-position. Increase in the size of the alkyl chain of the 9-amino derivatives with increase in the lipophilic properties of the compounds was no deterrent to staining of tumors.

Hata[10] (1932) stated that the staining and penetrating properties of dyes of the proflavine and trypaflavine type were associated with 3,6-diamino groups. Proflavine, or 3,6-diamino acridine, on oral administration does not stain the host's tumor while Acridine orange, or 3,6-dimethylamino acridine stains tumors diffusely.

Further studies with various monoamino and diamino acridines may determine the structural requirements for staining.

Summary. In our investigations on the structural nature of compounds that stain and retard tumor growth in mice, 331 acridine compounds (26 non-amino, 270 monoamino and 35 diamino) were administered orally to tumor-bearing mice in known percentages of dry weight of their food.*

Two hundred and four of them stained

* A table has been prepared showing the structural formulæ of the 331 acridine compounds studied, their source, percentage used, number of days fed, size of tumor and color at autopsy of the treated mice. The compounds are arranged as substituents of acridine based on the number of amino groups present on the acridine structure. Access may be had at the Wistar Institute to this table or to a microfilm of it.

tumor tissue (0 non-amino, 187 mono-amino and 17 diamino). The majority of compounds that colored tumors also brought about some retardation in their growth. The amount of retardation caused by 33 of the acridine compounds resulted in tumors that were 1/10 to 1/18 the size of those in untreated mice and 16 of them retarded tumor growth to such an extent that when the untreated mice died (14 to 16 days) with tumors measuring 8000 to 11,000 c.mm., the mice that had been fed these compounds were healthy and had tumors measuring 200 to 455 c.mm., or 1/20 to 1/40 that size.

The administration of acridine compounds did not prevent tumor growth or bring about regression of growing tumors. It simply slowed the rate of multiplication of tumor cells.

The majority of the 9-amino acridines that stain tumor tissue as well as retard its growth show dialkyl-amino-alkyl-amino chains in the 9-position. Analysis of the results from our studies on acridine dyestuffs and antimalarials has shown the importance of the presence of amino groups as substituents of the acridine structure for staining.

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GOUT: REPORT OF AN UNUSUAL CASE IN A YOUNG MAN

By Morton E. Berk, M.D.
ATTENDING PHYSICIAN, ST. FRANCIS HOSPITAL
HONOLULU, T. H.

(From the Department of Internal Medicine, The Medical Group)

Considering the prevalence of gout and our ignorance of much of its etiology and pathogenesis, there are comparatively few articles on the subject in current literature. Neuwirth[6] recalls that Daniel Sennert in the Sixteenth Century called gout the "physician's shame," *opprobrium medicorum*. The disease remains in that category, for there is still little we understand about this curious, crippling affliction.

Recently I attended a case of gout in which the bone and joint destruction were so far advanced that no parallel could be found in the literature examined. There were other aspects which were unusual enough to warrant a presentation of the case.

Report of Case. A. A., a 28 year old Filipino man, was admitted to the medical service at St. Francis Hospital, Honolulu, March 25, 1946. He was complaining bitterly of pain and swelling in his joints, particularly the distal interphalangeal joint of his left small finger. A few months previously his left hand had been caught in the drive-chain of a tractor. The pain had been slight until 4 days before admission at which time it became very severe. He had no complaints referable to other systems.

Past History. The patient is a Filipino born in San Fernando, Luzon, P. I. He recalled that before he was 11 years old he had recurrent bouts of pain in the metatarsophalangeal joint of both great toes. These continued at odd intervals through puberty and adolescence. Between these attacks he had neither pain nor limitation of motion. In 1939 he arrived in the Territory of Hawaii to work on a sugar plantation on the Island of Kauai. In May, 1940, because of pain and deformity in his right great toe, an operation[7] was performed in the plantation hospital and the contents at the base of the second phalanx were removed. These proved to be sodium urate crystals. Unfortunately the patient was not aware of the diagnosis of his ailment.

January 3, 1945, he was admitted for the first time as a private patient to St. Francis Hospital. His chief complaint was of pain in all the small joints of his extremities and in his spine. His oral temperature was 103.4° F. The positive clinical findings noted on the chart were confined to "swelling and pain of the small joints of the extremities." The ailment was diagnosed as chronic atrophic arthritis, and the patient was given supportive therapy plus sulfanilamide. He was discharged afebrile and free of pain on the 6th hospital day.

He was readmitted January 23, 1945 with swelling of his knees in addition to the previous complaints. This time his temperature was 102° F. He was put on the same regimen as before, and he was discharged on the 7th hospital day after the swelling and pain had disappeared. His diagnosis was rheumatoid arthritis and acute recurrent septic arthritis.

Family History. Non-contributory.

Dietary History. His diet had usually included milk, rice, vegetables, fruits, fish, and occasionally eggs or pork. Alcoholic beverages had rarely been taken before he left Luzon, and the amount he had had since his arrival in Hawaii was minimal.

Physical Examination. The patient was thin and undernourished; his face reflected anxiety and pain. His temperature was 101.4° F. All the positive findings were in the extremities. Most of the joints in the extremities were swollen, tender, and deformed; their movement was very restricted. Both hands deviated ulnar-ward. The distal interphalangeal joint of the right 5th finger was extremely tender, and purulent material (Table 2) was trickling from it. The right great toe was shorter than the left; and the scar from a previous operation was visible. Drainage of the infected finger was established and penicillin therapy instituted. A small "cyst" behind the right ear was opened, and the chalky material it contained proved to be sodium urate crystals. The Roentgen

ray photographs (Figs. 1 and 2) suggested gout, but because of the amount of destruction involved the diagnosis was not definitely ascertained until the crystalline material had been removed.

The patient was relieved of pain by colchicine. A purine-free diet, vitamins, and whole-blood transfusions rounded out the medical regimen. After surgical consultation the tophaceous material was removed from the left great toe and a partial amputation of the toe was done. The same procedure was carried out on the finger that had been infected. Improvement followed. Ambula-

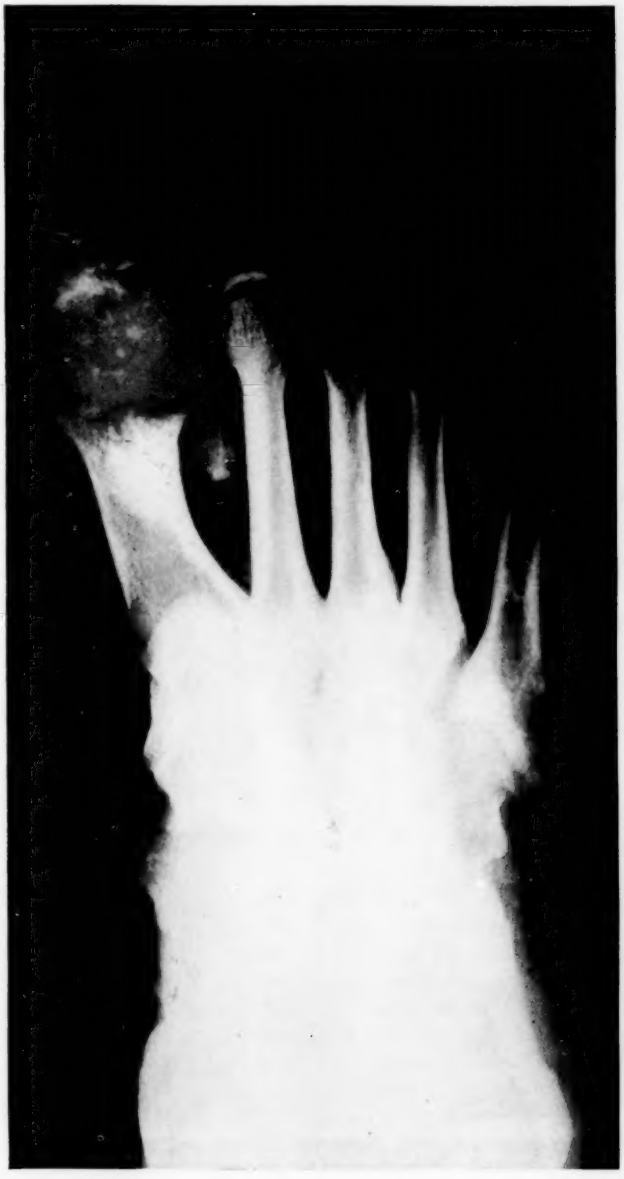

FIG. 1.—Roentgenogram of foot showing advanced destruction of bone and joint.

FIG. 2.—Roentgenogram of hand showing destruction of bone and joint with surrounding soft tissue swelling. Note punched out lesions in other digits.

tion was gradual, and the patient was discharged May 18, 1946 to the Maluhia Convalescent Home for further care.

Comments. This case has many aspects which contradict the traditional features of gout. It is rare indeed to see it in a young boy. Lansbury[3] includes age of onset as a diagnostic aid and points out that the first attack seldom occurs before the age of 35. Yet this patient was able to describe vividly gouty symptoms which appeared before he was 11 years old. In the 100 cases analyzed by McCracken *et al.*[5] the earliest onset was in a youth aged 19. There are no reliable statistics available as to the incidence of gout among Filipinos. According to the patient, there was no one else in his community on Luzon that suffered from the same type of ailment. Checking the records of the Queen's Hospital during the past 10 years for gout in Filipinos, I found but 1 case; but since a total of only 74 cases had been admitted (and diagnosed), this incidence is without significance.*

Sydenham's[8] classical description of gout appears to be the basis for the belief that this ailment is more prone to affect people who eat rich foods and imbibe freely of alcoholic beverages, though "poor man's gout" is also recognized. My patient had always been pathetically poor until he came to Hawaii. A basic food intake supplemented rarely by an alcoholic beverage composed his diet. Obviously diet had little influence on his contracting gout.

The tophus which confirmed the diagnosis was unusually located. It is common to find them on the ear margins, but this one was found behind the right ear and had the appearance of a sebaceous cyst.

The finding of hyperuricemia is taken as indicative of gout in those individuals who have arthritis. This patient on 5 different occasions had blood uric acid determinations under the outside normal limit of 5 mg. per cent. Bauer and Klemperer[1] point out that the uric acid concentration does not reflect the severity of gouty arthritis. The suggestion that old cases of gout and gouty arthritis may have lower levels of blood uric acid than early cases was confirmed in this patient.

The roentgenograms (Figs. 1 and 2) of a hand and foot showed extensive bone and joint destruction. On this basis alone the involvement might well have been interpreted as due to a malignant neoplasm. This interpretation was at first suggested by a competent roentgenologist after he had examined only the Roentgen ray photograph of the foot; but the presence of the lesion in two extremities made the diagnosis of malignancy unlikely. There are numerous punched-out areas visible in the bones, but no roentgenologist could be expected to make an unequivocal diagnosis of gout from these pictures. McCracken *et al.*[5] quote McClure and McCarty on this point and conclude with William's statement of a quarter of a century earlier that Roentgen ray is of little service in diagnosing gout, and it

TABLE 1.—NORMOURICEMIA AND BLOOD INDICES SHOWING INFLAMMATORY ELEMENT IN ACUTE GOUT

	Jan. 1945		Mar. 1946	Apr. 1946		May 1946	
	3	23	25	1	6	26	17
Blood uric acid, mg./100 cc.	..	..	3 3	3 0	4 0	3 6	3 7
Sedimentation rate, mm./60 min.	..	..	100	128	..	117	
Red blood cells, m./c.mm.	3 98	3 03	3 62	..	..	..	4 40
White blood cells/c.mm.	12,750	11,650	23,500	9900	7600		
Hemoglobin % (Sahli)	82 4	59 4	..	..	72 6	..	84 0
Differential blood count:							
Polymorphonuclears	93	90	91	72			
Lymphocytes	7	10	7	28			
Basophils	..	..	2				

* In June, 1945 the Territorial Board of Health statistics showed 47,863 Filipinos in the Territory of Hawaii.

may "lead one to arrive at wrong conclusions." Neuwirth[6] emphasizes this same point.

Linton and Talbott[4] show many interesting Roentgen ray photographs of cases which received surgical treatment. These exhibit more destruction than any others found in the available literature, and the results they obtained by careful removal of the tophaceous material is well demonstrated. Relief from crippling pain followed their surgical measures, and it was our desire to achieve the same goal in this case. This was accomplished with eventual rehabilitation of the patient.

It has not been sufficiently stressed in the literature that the acute arthritis due to gout is of an inflammatory nature. There is ample evidence to support this (Table 1). On each of the three admissions there was a moderate leukocytosis and a definite increase in the number of polymorphonuclear leukocytes. After the process had subsided, the leukocyte and the differential counts returned to normal. Although the sedimentation rate was over 100 mm. after 60 minutes when the patient was admitted in March 1946, it must be recalled that the patient did have a purulent infection in his finger. However, 1 month after admission the sedimentation rate was 117 mm., and there was no sign of pus at that time. This would suggest that the high sedimentation rate present at the time of admission was primarily due to the gouty arthritis. Further evidence was obtained by removing fluid from the left knee joint (Table 2). This fluid had a specific gravity of 1.023, with 250 polymorphonuclears per cu. mm.

TABLE 2.—EXAMINATION OF EXUDATES

	Culture from infected finger	Fluid from left knee
March 25:	Non-hemolytic Staph. aureus	Sp. gr. 1.023 250 cells (polys.) Culture—neg.
April 3:	Neg. for fungi	

In addition to the laboratory data, the temperature curve was above normal during all periods of pain. With colchicine and dietetic therapy the temperature (and pulse) returned to normal in a few days. There were a few exacerbations during the hospital stay, but they were mild in comparison to the attack which brought the patient in for treatment.

Discussion. Though there is still no adequate knowledge of the etiology of gout, certain negative statements can be made: (1) It is well established that hyperuricemia is not the cause of gout. Fiessinger[2] clarifies this stand and discusses the etiology from the standpoint of heredity and allergy. If gout ever becomes more readily diagnosed by the clinician, it is probable that hereditary influence will assume a more important aspect in our study of the disease. (2) Eating rich foods, particularly those high in purine content, is no more the cause of gout than is eating a high starch diet the cause of diabetes. The disease process must be present in some degree; then the diet may precipitate clinical symptoms. It is not only gout's etiology concerning which we are ignorant. Colchicum and its alkaloid derivative colchicine, the drugs of choice for the therapy of acute phases, act in a way unknown to us. Yet colchicum was used for treatment of joint attacks as long ago as the Seventh Century.[6]

The importance of the clinician recognizing the diagnosis of gout as a possibility in any case of arthritis cannot be overemphasized. That gout can mimic rheumatoid arthritis, polyarthritis due to rheumatic fever, acute osteoarthritis and acute arthritides due to bacterial infection should be obvious from the case history submitted. Since the prognosis and therapy for gout are widely divergent from other joint ailments, it is important for all physicians to consider gout seriously in their differential diagnosis of joint disease.

Summary. A case of gout is presented in which the following unusual features occurred:

1. The onset was previous to the patient's eleventh birthday.

2. The patient's diet was never very high in purines.

3. The roentgenograms displayed bone and joint destruction in excess of any found in the available literature.

4. Hyperuricemia was not present in several laboratory determinations.

5. A tophus was found behind the ear.

Criteria demonstrating the inflammatory nature of gouty arthritis are presented.

Physicians are reminded that gout can simulate other joint disease.

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PROGRESS
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UNDER THE CHARGE OF

JOHN E. GORDON, M.D.
PROFESSOR OF PREVENTIVE MEDICINE AND EPIDEMIOLOGY, HARVARD SCHOOL OF PUBLIC HEALTH
AND
W. LLOYD AYCOCK, M.D.
ASSOCIATE PROFESSOR OF PREVENTIVE MEDICINE AND HYGIENE, HARVARD MEDICAL SCHOOL
BOSTON, MASSACHUSETTS

THE PRINCIPLES OF VARIOLATION AS EXEMPLIFIED BY SUBCLINICAL IMMUNIZATION IN POLIOMYELITIS IN COOLER AND WARMER CLIMATES*

BY W. LLOYD AYCOCK, M.D.
AND
GILCIN F. MEADORS, M.D.†
BOSTON, MASSACHUSETTS

(From the Department of Preventive Medicine, Harvard Medical School)

REPORTS of groups of cases of poliomyelitis from tropical regions and from the Orient are likely to contain an excessive proportion of Europeans or Americans who not infrequently contract their disease while on even brief visits to these areas. This observation is remindful of the well-known epidemiologic principle that outsiders coming into endemic or seeded areas are, by reason of lack of immunity from previous exposure, more liable to attack than residents. But there is this important reversal: here the "outsiders" have come not from areas where the disease is less, but where it is *more* prevalent. Furthermore, the age of these patients is as a rule distinctly higher than that of native patients. An example is the report of Taylor on poliomyelitis occurring on the isthmus of Panama[53] from 1904 to 1942. In addition, even in the absence of reports of cases from tropical countries one is struck by the not infrequent development of the disease there in a visitor from this country. In conformity with many instances of this particular behavior of the disease, similar occurrences of poliomyelitis in British, American and Australian troops in the Middle East, Philippine Islands and in North China and Japan and in larger numbers (cited by Sabin[49]), has brought the phenomenon to the attention of a number of observers,[45,48,49] made it the subject of extensive investigation, and as well, the basis of far-reaching generalizations concerning the epidemiology of the disease. The apparent immediate cause of the phenomenon is a higher level of immunity in natives (developing earlier in life as a result of more

* Immunization of the prepared host with active virus as distinguished from immunization with prepared (attenuated) virus, vaccination.[206]

† Surgeon, United States Public Health Service.

frequent exposure to the virus in individuals while probably still under the protection of maternal passive immunity). On the one hand the areas where the phenomenon has been observed include in general those with poorer sanitation. On the other hand reasons are seen for believing that poliomyelitis in temperate climates has changed "from a sporadic to an epidemic disease" in the last 50 or 60 years coincident roughly with improving sanitation, with a shift in age distribution to older ages. These considerations coupled with the presence of virus in intestinal discharges, so much emphasized in recent years, have led to the formulation by some of the tenet that "poliomyelitis is the price we pay for sanitation."[48,49,60] In addition to its inference with respect to the mode of spread of the virus of poliomyelitis, this all implies that the disease in temperate climates in presanitation days behaved as it does now in the tropics. It also implies an exactness of correlation which does not seem probable between climate and improved sanitation extending all the way from Tasmania to Greenland. It further implies that climate *per se* is not a fundamental factor in the epidemiology of this disease.

Before deciding that man-made sanitation is responsible for such a difference in the behavior of the disease in warmer and cooler climates and has produced such a quick change from sporadic to epidemic occurrence in temperate climates, and before accepting these accompanying implications, it would be well to examine the record of the occurrence of the disease in point of time (also man-made), upon which the idea is based. Comparisons should be made with other diseases which exhibit the same behavior—and in which the various elements involved are more amenable to measurement. Finally it is necessary to seek in the information thus accumulated an explanation which might apply not to just one of its members, and with an element of doubt, but with more certainty to all of the group of diseases which exhibit the identical phenomenon.

Change From Sporadic to Epidemic? That a disease is "on the increase" has always been one of the most potent arguments in the launching of campaigns, but at the same time one of the most difficult of epidemiologic features to establish. There are many examples of apparent increases in disease which are clearly the result of the separation (frequently by the keen mind of a single observer) of clinical entities from larger classifications in which they had been included—or better, actually submerged. It would be difficult today, for example, to make any comparison between the actual incidence of appendicitis in the periods before and after Reginald Fitz. Again some advance in administrative, diagnostic, or therapeutic procedure may initiate or increase the recording of a disease not previously coming even within clinical recognition (*e. g.*, virus pneumonia and non-tuberculous pulmonary calcification).

The apparent increase in the occurrence of poliomyelitis in the last 20 years as compared with the preceding 20 year period is evidently attributable to increase in the recognition and reporting of nonparalytic (or preparalytic) poliomyelitis, which was raised to a new level in 1927.[9a,37] There has been no such increase in the more stabilized reporting of deaths.[11]

Indications of the occurrence of poliomyelitis can now be traced to the earliest times in the records and preserved remains of human history, but in medical literature it was not until the beginning of the 19th century that it began to emerge as a disease entity from the heterogenous group of accepted but little noticed "crippled and deformed."[16] It was through case descriptions of a particular kind of crippling which included some attempted association with one or another specific cause, that the concept of an epidemic infectious disease began to take form. Underwood's "Debility of the Lower Extremities" is generally regarded as the first of these: "Not a common disorder, attacking children seldom under 1, or

more than 4 or 5 years old, . . . in the instance of teething or of foul bowels."

At any rate, this and subsequent more definitive descriptions were forerunners of Heine's monograph in 1840, which, although dealing primarily with the orthopedic management of various paralyses, included the clinical course as well as his speculations on the etiology and pathology of the disease. He called it an affection of the "central nervous system, namely of the spinal cord, of an irritative and congestive sort"—good beginnings for present-day knowledge, but not until his concept had survived years of challenge by such theories as "essential infantile paralysis" did it lead to the next advance. Strümpell, in 1884, first suggested infection. In 1887, Medin observed the epidemic nature of the disease in Stockholm, and, in 1894, Caverly studied an outbreak in Vermont, added non-paralytic forms of the disease, and laid down the important epidemiologic features; and finally, the exhaustive studies of the 1905 epidemic in Sweden by Wickman carried the earlier work to the concept that the infectious agent was widespread largely through mild, abortive, or subclinical infections with the frank disease an exceptional outcome of the exposure to the infectious agent.

Taylor's epidemic[55] of 7 cases of paralytic poliomyelitis in the village of Cherryfield, Maine, in 1896, surpassed the great epidemic in New York City in 1916. Caverly's epidemic in Rutland County, Vermont,[15a,b] which occurred 53 years ago was surely as epidemic in that population as any we have known later. To be sure, Caverly's epidemic extended only as far as he could travel with a horse and buggy, but numbers of cases which had occurred outside this area in the same outbreak became known later as well as cases which had occurred in earlier years. The earliest case in Vermont actually verified by examination by one author had occurred in 1852—almost back to Heine.

Hirsch had to write in 1880 that "Historical inquiry . . . into the relations of typhoid in time and place can only cover a somewhat short period with any exactness, a period counted by tens of years." If typhus abdominalis melts into an undifferentiated group so quickly when we try to trace it back, when we ourselves can remember many cases of poliomyelitis which had not yet been separated from "the crippled and deformed," it is no wonder that records of epidemics of poliomyelitis can be traced no further back than those of typhoid fever. One can judge—from the fact that even today many cases of poliomyelitis are the first in the experience of the physician, and many epidemics the first for the health officer of the community—that the papers of Jacobi (Mary Putnam)[32] and Sinkler[51] in Philadelphia in the 70's, describing as they did all the types of cases seen today, certainly represented an experience with far more than a "sporadic" occurrence of the disease. Heine's monograph in 1840 indicates a familiarity with various types of cases equal to that of an orthopedic surgeon of today. Colmer's report of an epidemic of poliomyelitis 2 years later in the Parish of West Feliciana in Louisiana was a large epidemic for that population. And Underwood, in 1789, was not describing a rare experience in his description of "debility of the lower extremities" when he wrote that when 1 leg is affected the "above means (his therapy) in 2 instances out of 5 or 6, entirely removed the complaint; but when both have been paralytic, nothing has seemed to do any good but irons to the legs."

Van Riper fixes the change from sporadic to epidemic in various parts of the world with a precision which is believed to reflect the man-made record of poliomyelitis more than it does its actual occurrence in the past, when he writes, "Prior to 1870 poliomyelitis occurred only as a sporadic disease of the very young child. During the 1870's in Europe, the 1880's in Scandinavia, the 1890's in the northern part of the United States, and the 1930's in the southern part of the United States, poliomyelitis took on a new character—occur-

ring in epidemic proportions."[60] One of us remembers 2 individuals in his small home town in Georgia who had always been known only as "cripples" but who, after the occurrence of poliomyelitis in 1 of their children many years later, were both diagnosed as having had poliomyelitis in August, 1889—an attack rate in that small population equal to that of the New York City 1916 outbreak and coinciding in time with Medin in Sweden.

AGE DISTRIBUTION. The age distribution of "the immunizing infections of childhood" is a reflection of both opportunity for exposure and immunity existing at the time from previous exposure.[1d] One determinant in the rapidity and extent of exposure to and development of immunity from exposure is density of population. Perhaps the most clear-cut example of this is the occurrence of measles at younger ages in urban populations than in rural populations in the same region. The disappearance to a large extent of the urban-rural difference in the age distribution of measles in many parts of the United States in more recent years has been attributed to the "urbanization" of what has been designated as rural populations. Such factors as increased facilities for transportation and consolidation of schools are generally considered as contributory.

Clinical observation alone has been sufficient to establish measles as one of the "immunizing infections of childhood" without benefit of either tests for detection of virus or of immunity resulting from infection. If only cases of measles with complications (middle ear, pneumonia, or post-measles encephalitis, for example) were recognized, that is if the rash had never been seen, the distribution of "measles" would only dimly reflect the dissemination of the virus and could not be fitted into any epidemiologic pattern in the absence of tests for virus or for immunity. The conception of the epidemiology of measles would be as vulnerable to challenge as has been the epidemiology of those diseases where infection and the development of immunity is largely subclinical. Both Frost and Wenstedt many years ago interpreted the age distribution of poliomyelitis and especially the more rapid decline in cases with age in urban than in rural populations as likewise indicative of widespread subclinical immunity which accumulated more rapidly in urban than in rural populations. From a comparison of the age distributions of measles, diphtheria and poliomyelitis in urban and rural populations, it was concluded that the virus of poliomyelitis attained a distribution equal to that of measles and diphtheria and it was inferred that the mechanism of infection was the same for the 3 diseases, the essential epidemiologic difference being the frequency with which those exposed to the infectious agents developed clinical disease or subclinical infection. In measles there is a lack of evidence that subclinical infection occurs, but there is the possibility that exceptional individuals, giving no history of measles are presumably immune since they fail to develop the disease even upon repeated exposure. It may be that they were actively immunized through modified measles (unrecognized) resulting from exposure at a suitable time during the short period of their maternal passive immunity. In diphtheria subclinical infection is roughly 10 times that of clinical infection, and in poliomyelitis more than 100 times as great.[1a]

In warmer climates even in less densely populated areas, the age distribution of certain of these diseases notwithstanding lower incidence indicates that dissemination of their infectious agents with resulting immunity takes place even more rapidly and extensively with reference to age than in cooler climates. Doull[21] has shown that in diphtheria, scarlet fever and poliomyelitis there is a higher concentration of cases at younger ages in tropical and subtropical than in cold climates, a phenomenon interpreted by him as an indication of earlier and more widespread immunity in warmer climates. As he said, subclinical infection is regarded as being

more frequent in these climates than in colder latitudes where clinical forms are much more common.

Even within this country the same climatic difference can be discerned. It was noted, in 1929, that in spite of lower incidence of poliomyelitis in the South, the age distribution indicated "that immunization, as in diphtheria, is accomplished . . . with a rapidity equal to that in the North; this may in turn be regarded as evidence that the virus spreads to the same extent in the 2 parts of the country. It should be remarked that the close approximation between the age distribution in New York City and in the Southern States, the latter comprising a comparatively rural population, suggests that the virus may spread with even greater rapidity in the South from the point of view of age, and that the possibility of infection with the virus earlier in life in the South may be a factor in the production of the relatively more extensive subclinical immunization in the southern population. It should be stated, however, that there is no positive evidence that infection at earlier ages (*per se*) tends to produce subclinical immunity, excepting (by analogy with measles and diphtheria) possibly during the first few months of life, when there may be a temporary immunity derived from an immune mother which, if infection occurs during this short period, might bring about an active subclinical immunity."[1b]

Thus, insofar as age distribution may be taken as a reflection of the rapidity and extent of virus dissemination, it would appear—from the fact the patterns are the same—that the mechanism of dissemination (and immunization) are the same in a group of diseases including diphtheria, scarlet fever and poliomyelitis, and that all 3 diseases exhibit the same evidence of earlier exposure in warmer than in cooler climates. The effect of an evident decrease in dissemination of the diphtheria bacillus (usually attributed to artificial immunization) in northern United States and Canada in recent years, on the age distribution of diphtheria, has not been examined.

Immunity. The epidemiologic inferences from studies of the age distribution of these diseases in respect to widespread dissemination and immunization (more largely subclinical, as so ably set forth by Wickman in his classic monograph on poliomyelitis) have been amply borne out chiefly by actual tests for immunity and in a more limited way by tests for detection of the infectious agents. Many Schick test surveys in urban populations have revealed immunity which increases with age to the degree which was fully to be expected from the decrease in incidence of the disease with age. The less rapid development of immunity and to a lower total level than that observed in urban populations has been determined by Schick test surveys in rural populations, notably the study of Kidder[36] in Vermont.

The results of extensive Schick test surveys in a number of tropical countries where clinical diphtheria is relatively uncommon have consistently shown higher proportions of immunes than are found in even more congested populations in northern areas.[21,22b]

Tests for immunity to poliomyelitis, although carried out far less extensively than tests for immunity to diphtheria, have been done to a sufficient extent to indicate a similar population immunity. Neutralization tests on persons at different age groups in urban and rural populations revealed immunity corresponding closely with that to diphtheria as indicated by Schick tests.[8a] Numbers of tests in various parts of the world have indicated immunity in adults as extensive as immunity to diphtheria. Going to warmer climates, 20 of 21 adults in Atlanta, Georgia, possessed neutralizing antibodies.[8b] Hudson and Litterer found the same in Nashville, Tennessee.[31] A high proportion of sera from the Fiji Islands neutralized the virus.[1e] A similar high immunity has been found in adult natives of Liberia.[30a,b] Immunity has also been shown in high proportion of small groups of adults in

China[34,40] and in Puerto Rico.[52] It is to be noted that the extent of immunity in adults in warmer climates, even though the population may be more sparse, is equal to or exceeds that of adults in more temperate climates. Doull, Hudson and Hahn[23] testing adults of the Philippine Islands found evidence of immunity to diphtheria, scarlet fever and poliomyelitis in a higher proportion of the sera than that usually found in temperate climates.

That the differences under discussion between cooler and warmer climates are not due to variation in sanitation with latitude but are in reality determined by climate itself is perhaps most strikingly suggested by the occurrence of the same differences in diphtheria between 2 cities in the same latitude—Santos and São Paulo, Brazil, not 50 miles apart but one situated at sea level and the other at 7500 feet altitude.[22a] Altitude produces the same effect as latitude. The same difference has been observed in poliomyelitis in Bogota and at sea level in Venezuela.[39b]

The parallelism between latitude and climate is, of course, not an exact one. In addition to altitude other factors such as ocean currents account for marked variations, as for example the effects of the Gulf Stream. On the North American Pacific Coast the more precise variations with latitude seen in the interior do not occur. A number of infectious diseases ordinarily conforming closely with latitude in respect to such features as incidence or seasonal prevalence are aberrant, partaking of some features characteristically southern and others characteristically northern. That the "climate" of the Pacific Coast possesses both northern and southern features is suggested by the fact that Timothy and Bermuda grass, respecting completely their northern and southern ranges elsewhere, overlap on the West Coast. In this connection it may be pointed out that poliomyelitis in Japan corresponds closely with poliomyelitis in the southern United States in respect to incidence, age distribution and seasonal prevalence.[45]

Another related phenomenon common to this group of diseases is that of maternal passive immunity. Since in adults immunity is high, passive immunity in infants, as would be expected, is likewise high. This has long been established in diphtheria as lasting from 3 to 6 months by antitoxin measurement and has been demonstrated in poliomyelitis by virus neutralization tests.[8c] The duration of maternal passive immunity in infants has not been so precisely established in poliomyelitis, but the close parallelism in the diminished incidence of the disease in infants under 6 months of age with diphtheria suggests that there is a similarly close correspondence in maternal immunity in respect to duration. This is borne out by the absence of immunity in a high proportion of children over 6 months but under 3 years of age (in temperate climates).

Just at the time when the results of immunity tests so generally accepted and widely used as a measure of the dissemination of other infectious agents were reaching the point of full verification of the implications of age distribution in respect to the pattern of infection with the virus of poliomyelitis, the epidemiologic significance of immunity in poliomyelitis was thrown into the shadow by the advancement of the theory that immunity was the result not of exposure to the virus but of "physiologic maturation." The idea that immunity, as shown by virus neutralization tests and even diphtheria antitoxin, was a spontaneous accompaniment of aging, was given considerable editorial emphasis under such headings as "Panimmunity," "Automatic Environmental Vaccination," "Upsetting Immunologic Tenets," and "Phylogenetic Immunologic Recapitulation." Further doubts continue to be raised, doubts introduced through discrepancies[17,12] in the neutralization tests but which are believed to be of the same order as less noticed discrepancies observed in immunity tests in other diseases. Such discrepancies led, as late as 1939, to the statement that "Polio-

myelitis antibody is not a result of exposure to or infection by the virus of epidemic poliomyelitis."[13]

In spite of all the questioning of the "epidemiologic specificity" of immunity, the immune reaction has never ceased to hold first place in the laboratory as a test for "etiologic specificity." The circumstances attending most of the "transmissions" of poliomyelitis to rodents are similar to those under which spontaneous but highly latent rodent virus diseases have been uncovered. Cross-immunity has been the main criterion for differentiation, as in the well-known examples of the 2 types—the Lansing strain of poliomyelitis and Theiller's virus. Whether the close biologic relationship between the infectious agents of 2 different diseases suggested by cross-immunity is an indication that both were derived from the same stem, whether one has arisen from the other by mutation, or whether their antigenic components only happen to have the same or a similar configuration, may be an interesting academic question but may not be of epidemic significance. There is evidence of cross-immunity between *T. pallidum* and *T. pertenue* and "There has been much speculation on the possibility of syphilis having been derived from yaws or *vice versa*, but creditable evidence that this has ever occurred within historic times is lacking."[59]

Reëxamination of the question of transmission of poliomyelitis to rodents in the light of spontaneous rodent viruses which cross-immunize with poliomyelitis virus should go far to clarify some of the epidemiologic implications of "rodent poliomyelitis"—particularly those relating to extrahuman reservoirs in poliomyelitis. Furthermore, 1 of these immunologically related but etiologically unrelated rodent viruses, as suggested by Turner, in the case of *T. cuniculi* in syphilis or yaws, might (in the vaccinia sense) actually constitute a vaccine against poliomyelitis.

It was not until Olitsky and Findlay's[44] neutralization tests—done in the face of the strikingly high incidence of poliomyelitis in troops as compared with tropical natives and indicating high immunity in native children—that the implications of the earlier work on immunity again have entered heavily into conceptions relating to the epidemiology of poliomyelitis, and have finally attained a place in the minds of many as a true indication of widespread exposure to the virus—as widespread as is exposure in the other "immunizing infections of childhood."

Not only diminished incidence under 6 months of age but passive immunization in diphtheria and such procedures as convalescent serum protection against measles are all reasons for believing that maternal passive immunity is protective. Furthermore, such phenomena as production of modified measles and toxin antitoxin immunization and vaccination with immune serum-virus mixtures indicate that exposure to active virus under protection of maternal immunity or other protective influences may result in active immunization (subclinical).[2] Maternal immunity in offspring of immune mothers in a rodent poliomyelitis has been shown to protect against disease but to allow active immunity on injection of fully active virus. Virus injected early in the period of maternal immunity produced no effect; when injected later in the period of maternal immunity, the mice developed persisting active immunity (but without disease); when the virus injection was given still later, all succumbed to disease as was the case with the young of nonimmune mothers when given active virus at any age.[20]

In temperate climates, as shown by the several immunity tests and as reflected in the age distribution of disease, immunity of the population is at a high level during the first 6 months of life, at its lowest level at 1 to 2 years of age, after which it increases steadily and in accord with opportunity for exposure to the infectious agent until the high level which had existed at birth is reëstablished in adult life.

In tropical areas not only does the age

distribution of the disease suggest that exposure to virus more often approaches the early period of life when there is high maternal immunity but the neutralization tests of Olitsky and Findlay[44] in such an area suggests that the gap between maternal passive and acquired active immunity may be lacking. In 1946, they found that all of 72 sera from "healthy" natives of British West Africa from 1 to 40 years of age (8 were under 10) neutralized the MEF1 strain of virus. This strain of virus was isolated from a case in the Middle East Forces of the British Army and was shown to cross-immunize with the Lansing strain. Normal human serum possessed neutralizing antibody to the latter strain of the same order[59] of that repeatedly demonstrated against a number of monkey strains of virus.[44]

A series of neutralization tests in 1933 and 1934, the interpretation of which was not entirely clear at the time,[1e] were done in the course of a survey of immunity to poliomyelitis in tropical island populations, one object of which was the finding (so far unsuccessful) of a population free of immunity, (needed at the time as a convincing answer to the challenge of spontaneous physiologic maturation). The possibilities of finding such an isolated population are not exhausted, but the occurrence of a "virgin soil" type of outbreak on the Island of Nauru, in 1910, restricted to the native half of the population suggests that a survey previous to that time doubtless would have uncovered an absence of immunity. The "isolation" of this group at that time is likewise suggested by the wide occurrence of leprosy in the same group following a clear history of its introduction a year later. Of sera from 32 natives of the Fiji tested against an American and an Australian virus, 26 of the 27 from adults showed the presence of antibodies. Sera of younger individuals, 11, 11, 12, 16 and 18 years old, all neutralized the virus, the 16 year old partial in 1 test but complete when retested. Of 27 sera from "normal" natives in Samoa, 23 completely neutralized and 4 gave evidence of partial neutralization, the animals inoculated with the serum-virus mixture developing disease after incubation periods of 17, 18, 20 and 21 days, as compared with 7, 7, 8, 9 and 14 days in control animals. These 4 sera were from children 3, 4, 5 and 5 years of age. Nine other sera from children under 10 years of age completely neutralized the American laboratory strain of virus (isolated in Vermont in 1921). Though prolonged incubation periods had been suggested as representing partial immunity (in the serum under test),[9b] this finding in respect to the sera of 4 children in the Samoan group was not considered sufficient to establish a difference from the usual immunity status of younger children in temperate climates (a relatively higher proportion of non-immunes). Subsequent observations have made it clear that prolonged incubation periods in such tests are indicative of the presence of antibody in serum.[50] In view of this and in the light of the results in the larger series of Olitsky and Findlay, these tests can be interpreted as indicating that the hiatus of immunity in younger children tends likewise to be filled in Samoa. The occurrence of partial neutralization suggests that active immunization was still in process of development at the time tests were done, a finding in keeping with the observation that active immunity can be initiated only in the later stages of maternal immunity when it is on the decline.[20]

Virus Detection. That immunity as revealed by the several tests is a result of previous exposure to the infectious agent would no longer appear to need argument. Suffice it to say that wherever tested, either by clinical observation or by virus detection, the development of immunity has been in accord with opportunity for exposure. To be sure "control" areas (where virus and immunity are both absent) have not been found; but truly isolated populations insofar as human-to-human transmission is concerned are not to be found easily in the present day

The last thoroughly studied "virgin soil" epidemic of measles (Faroe Islands) occurred just over 100 years ago. Perhaps the development of immunity to yellow fever, only in areas where there is opportunity for exposure and never in areas or in persons where previous exposure to the virus could be excluded, should suffice as "proof" of association between the presence of virus and of immunity.

In diphtheria Morales and Mandry[42] have actually put to test the epidemiologic "hypothesis" that subclinical immunity is a result of exposure to the infectious agent in Puerto Rico. They followed a group of 194 native Schick positive school children with frequent throat cultures for diphtheria bacilli. At some time during the period of the study virulent bacilli were demonstrated in 26 individuals and in 168 they were never found. Of the 26 who harbored virulent organisms, 24 became Schick-negative and 151 (90%) of those who were never shown to harbor bacilli remained Schick-positive. Fewer data are available on scarlet fever, but wherever tested, to quote Doull, "every item cited in support of the explanation offered as to diphtheria holds true."[21]

As stated earlier, clinical observation alone affords a satisfactory demonstration of both the extent of spread of the virus of measles and of immunity resulting from infection with it. When immunity tests and virus detection are added, evidence is likewise afforded that the extent of dissemination of the subclinically immunizing viruses and of immunity from infection with them is of the same order as that in measles and, therefore, of an extent over the entire world only known to take place through person-to-person contact.

The disappearance of cholera in the United States within about 20 years after a widespread occurrence following its introduction in 1830 (described under the somewhat odd title "The Conquest of Cholera"[17]) soon followed when we began to get away from the river life of the times on the Mississippi and the Ohio. It well illustrates the precarious method of transmission chosen by this disease. Cholera is now a problem only in restricted areas where optimal conditions for transmission exist. Typhoid fever has been able to maintain an existence only by reason of the added mechanism of chronic carriage. It too, however, is steadily diminishing, as with increase in sanitation the chances of continuous transmission are decreasing to the point where the failure of recruitment of new chronic carriers is causing it to lose its grip. Typhoid Mary is positively credited with only 50 odd cases in a lifetime, a feat which can be accomplished by a single case of measles in a day. Fecal transmission—an accident of hygiene—is not to be compared with the mode of transmission which has been described as ordinary, unavoidable, and the probable irreducible human contact, chosen by the upper respiratory infections, through which these infections enjoy a spread in the population to the point of saturation.

By the time the period of controversy which followed the challenge of "maturation" was subsiding, emphasis in epidemiologic research in poliomyelitis had shifted to attempts to establish the pattern of virus distribution through its actual detection. The "rediscovery" of virus in intestinal contents reopened the whole question of mode of spread of the disease with the result that exhaustive attempts were made to detect the virus not only from numbers of human sources but from a wide variety of non-human sources. There was a great emphasis on the detection of extrahuman virus reservoirs, which was doubtless prompted in part by the temporary dismissal of the large accumulation of evidence pointing to widespread subclinical infection and further stimulated by a resulting reëmphasis of the long-known inability to demonstrate the criteria usually sought to establish contagiousness between clinical cases—the occurrence of disease in those associated with the sick. It became the fashion to "discard all previous conceptions"—the

force of the combined studies of Caverly, Wickman, Frost and Flexner—which had "dominated the scene too long" and to test any hypotheses by the sole method of virus detection; a phase of research which reached its height in a title as inclusive as "attempts to recover poliomyelitis virus from fruit, well water, chicken cords and dog stools."[56] The confidence with which the hunt for extrahuman virus reservoirs was on is well illustrated by an abstract of the just mentioned article which seems to assume that failure to find the virus was only a failure in technique when it says: "Although the virus may have been present in the specimens tested, its existence could not be demonstrated when either the eastern cotton rat or the *Macaca mulatta* monkey was used as the test animal."[43]

During the period when immunity tests were largely relied upon as an indication of previous infection with the virus, relatively few attempts at actual detection of virus were undertaken. In view of the limited scope of researches which could be undertaken at the time, there was little chance in the first place of carrying out experiments on a sufficiently large scale. In the second place the end-point of virus infection as indicated by immunity tests rather than momentary harborage, as revealed by virus detection, seemed to be a better means of arriving at a conception of the actual extent of virus dissemination. During more recent years the detection of virus in intestinal contents has received far greater emphasis. Although it is clear that virus may be found in intestinal contents for a longer period than in the oropharynx where it has been detected during the 1st week of the infection (the period coinciding with the infectious period according to available evidence),[7,9b,14] it cannot be affirmed that it is more often present in intestinal contents. The large literature which has accumulated relating to the detection of virus in intestinal contents and in extrahuman sources will not be reviewed here, but it may be said that the results of such tests as well as renewed detection of virus in upper respiratory passages[29,35] have gone far to corroborate a virus distribution in the human such as would result, not from any extrahuman reservoir but from person-to-person contact as had been indicated previously both by studies of the age distribution of the disease and by the application of immunity tests. In this connection the work of Francis and his co-workers[47] and a recent study of Zintek[62] on the dissemination of virus within a family, and of Howe and Bodian, who found virus in throats in juvenile family contacts and in throats of healthy children from a neighborhood playground,[28] may be cited.

It has been calculated that a diphtheria carrier rate of near 1% with an average duration of carriage of approximately 1 month[26,61] is sufficient to account for immunity as indicated by the Schick test at any age group.[8a] It was stated a number of years ago that "while no statistics are available concerning the healthy carrier rate in poliomyelitis, the virus has been detected in healthy persons. When we remember the relatively small number of attempts which have been made to detect the virus and the uncertainty of the technique by which this has been accomplished (it is not even readily transferred to the monkey from the spinal cord of known cases), the occasional reported finding of the virus in the upper respiratory passages of healthy persons might well be indicative of a healthy carrier rate not unlike that of diphtheria. When we consider, then, not only that the extent of immunity to poliomyelitis is the same as for measles and diphtheria, but that the rapidity of its development varies in the same way with concentration of population, we have evidence that both the extent and rapidity of the spread of the virus of poliomyelitis are the same as in measles or diphtheria."[1e] The more recent tests for detection of virus on a large scale such as those of Francis amply bear this out. It therefore may be said that the carrier rate in poliomyelitis (something

like that of diphtheria) would likewise account for immunity to this virus at different age groups of the same order as that of immunity to diphtheria.

Trask and Paul,[46,57] who with their co-workers had used this method extensively, pointed out in connection with their detection of poliomyelitis virus in sewage and in intestinal contents, that the procedure used was in a number of ways highly selective for poliomyelitis as, for example, initial treatment of the inocula with ether on the one hand and the use of the monkey as test animal on the other. They actually "detected" tubercle bacillus in sewage as an incidental finding and stated that other "pathogenic bacteria, measles, mumps, etc., could have infected our monkeys without our knowing it." It is, then, not possible to attach proper epidemiologic significance to the presence of poliomyelitis virus in intestinal discharges in the absence of comparable information on other viruses. For example, is the virus of measles present in feces? As a matter of fact, lesions in the intestinal tract have been found in a number of diseases clearly not transmitted by intestinal discharges which make it likely that their viruses may also be present in intestinal contents and presumably in sewage. Koplik spots have been found in the colon in measles,[19] as have been characteristic cellular changes in varicella.[33]

In addition to such considerations as these, Paul and his co-workers have repeatedly called attention to the danger of basing epidemiologic inferences on this experimental finding alone: "But perhaps the most important question of all is, what does the finding of the virus mean insofar as the spread of poliomyelitis is concerned? In answer one could say that it is not evident from this work whether or not the presence of poliomyelitic virus in sewage is a direct or even an indirect link in the chain which usually or even occasionally leads this infectious agent from 1 patient to another in this disease. Our report merely calls attention to the fact that during urban epidemics of the disease the local sewage may contain this virus."[46]

Nonetheless this single laboratory finding seems to have been largely responsible for a very general shift from previously long-accepted conceptions to the belief that "the epidemiology of poliomyelitis may be considered to be similar to that of other enteric infections in which the causative virus is much more widely disseminated in the community during an epidemic than at other times."[24]

It is this belief which forms the crucial link in the chain of argument that the lower incidence of poliomyelitis in warmer climates and its supposed increasing incidence in cooler climates is a matter of sanitation.

An ideal situation for studying the spread of disease would be afforded by an outbreak following the importation of a single case into an isolated and previously uninfected locality. But such an opportunity is seldom presented to the epidemiologist. Under actual conditions it is practically impossible to determine whether a given case is the first occurrence of the disease, or to group those cases which are related. Multiple cases in families present perhaps the nearest approach to epidemiologically connected cases.[3] Chapin,[18] Hill[27] and Frost,[25,38] all chose familial aggregation for studies relating to the manner and the extent of dissemination of such diseases as measles, diphtheria, scarlet fever and poliomyelitis. The results of all the studies reviewed in this paper amount to hardly more than a geographic extension of the combined findings of these 3 great epidemiologists. Their studies supported the view that the "tendency to run through a family" was the same with all these infectious agents, and that the differences in familial aggregation of cases are due to differences in the frequency with which clinical or subclinical infection is the result of exposure.

Since the patterns of virus dissemination and immunity are essentially the same in the members of this group of diseases,

poliomyelitis, diphtheria and scarlet fever, it is believed that the relative frequency with which clinical disease and subclinical immunity results from infection with the different infectious agents cannot be accounted for in general by "immunity from previous exposure" but are actually due to different autarceologic factors in the different diseases. The question has been little studied in diphtheria and scarlet fever. In poliomyelitis there are a number of selectivities seen in the occurrence of the frank disease in the few of the many exposed to the virus which indicate that this "complication" of the ordinarily subclinical infection is determined primarily not by circumstances of exposure to the virus but by autarceologic influences. The notable example—already being taken into account in the prevention of numbers of cases of a particularly distressing and highly fatal form of the disease—is bulbar poliomyelitis following tonsillectomy and adenoidectomy.

Though the frequency with which the different infections are clinical or subclinical may be highly individualized in general, as the result of the operation of different autarceologic factors in the separate disease, they all possess a corresponding reduction in the frequency of clinical disease as compared with subclinical infection as warmer climates are approached. The evidence for a similarly more rapid and extensive dissemination of all the infectious agents in warmer climates suggests that earlier infection in warmer climates and hence more often under the protection of maternal passive immunity may afford the explanation for this phenomenon which the 3 diseases have in common.

Mechanism of Increased Virus Dissemination in Warmer Climates. A mechanism which may account for the dissemination of virus at a more rapid rate and more extensively in the tropics is suggested by a study of amplitudes of seasonal variation in a number of diseases in cooler and warmer climates.[5,10] In respect to amplitudes of seasonal variation the curves of seasonal prevalence of a number of diseases in cooler and warmer climates all fit closely one of 3 curves. With certain exceptions bacterial diseases which spread directly from person to person have an amplitude of low order. Another group including diseases transmitted by intermediary hosts have an amplitude of higher order; and a third group, virus diseases, exhibit a still greater amplitude in seasonal variation. It was considered significant that the 3 curves in respect to amplitude occur in exact multiples. The reason for the exact multiple relationship was believed to be the simultaneous influence of season on 1, 2 or 3 factors involved in the occurrence of diseases of the respective types. It was suggested that 1 of the factors involved in the group of diseases exhibiting an amplitude of a higher order was, in addition to seasonal variation in susceptibility to disease upon exposure, a seasonal variation of the same period and phase in multiplication and hence in spread of the virus. For example, under this conception it could be reasoned that the virus of poliomyelitis requires host conditions of warm weather (or warm climate) both for disease production and for multiplication and hence propagation. Such a conception under which this virus could be active for only a brief part of the year in more temperate climates and throughout the year in warmer climates, could account for the more rapid and extensive dissemination of the virus in warmer climates.

Thus the reason for one of the puzzling paradoxes of poliomyelitis—the fact that it is a disease of warm weather but of cool climates—finally begins to come to the surface. For many years it has been evident that the conventional explanation for seasonal prevalence of disease—a corresponding variation in the mechanism of transmission—could hardly account for the seasonal prevalence of poliomyelitis in the face of this feature of the disease.[15] Nonetheless, adhering to the old doctrine that contact diseases are diseases of winter

because of crowding, the seasonal prevalence of poliomyelitis has always been the point of departure for opposed hypotheses.

Under the same doctrine the view originating largely in carrier studies during World War I, that an increased carrier rate is always the forerunner of an increase in meningococcus meningitis, in spite of many discrepancies in the evidence, had come to be generally accepted. During World War II an extensive meningococcus carrier survey was conducted over a period of 4 years by Dr. J. H. Mueller under the Board for the Investigation of Influenza and Other Epidemic Diseases, Office of the Surgeon-General, United States Army. An analysis of the carrier rates, especially Type I meningococcus, the type found to be the cause of practically all cases, revealed no seasonal variation in carriage which could be associated with seasonal variation in the disease. As a matter of fact, carrier rates in summer were as high as in winter when the disease prevailed. This observation points to variation in frequency of clinical disease in carriers rather than to any variation in transmission of the meningococcus as the determining factor in the seasonal prevalence of meningitis, and suggests seasonal "predisposing" factors as the major determinant of meningitis in meningococcus carriers.[4,5] Little information is available on the year-around prevalence of other infectious agents. But if the findings of the virus of poliomyelitis in sewage only in the poliomyelitis season[41,57] or a prevalence of carriage in summer as suggested by the seasonal prevalence of bulbar poliomyelitis following tonsillectomy[19] can be compared with the seasonal prevalence of meningococcus carriage based on some 50,000 throat cultures, there is the suggestion that the meningococcus is capable of multiplication and propagation the year around, while multiplication of the virus of poliomyelitis is restricted in respect to season. This suggests that seasonal (or climatic) changes influence the suitability of the human host as a "medium" for the growth of the virus. this view is consistent with the fact that meningitis exhibits an amplitude in seasonal variation of a low order, and poliomyelitis one of high amplitude in temperate climates and lower amplitude in warmer climates.

A review of the observations which form the basis of this paper serves to lend strength to the view that the spread of the virus of poliomyelitis is extensive and, as has been stated, a function of ordinary, unavoidable and irreducible contact. It furthermore emphasizes that infection with this virus is preponderantly benign —the one serious sequel of infection, paralysis, occurring in only a small fraction of those exposed. In the tropics the evidence indicates that in terms of parasitism adaptation between virus and host is even more nearly balanced. Widespread exposure to the virus, more frequently during the persistence of equally widespread maternal immunity, apparently results in more frequent modified infection (but still with propagation of virus) with resultant active immunity to be passed on to the offspring for repetition of the cycle. Thus we can say that we have achieved a high degree of commensalism with the virus (or the virus can say that it has achieved a high degree of commensalism with us).

Immunization (as in smallpox) would appear to be the approach to control of an infection as widespread as is poliomyelitis. The feasibility of vaccination against smallpox (carrying with it a certain hazard as would be expected in any prospective procedure in poliomyelitis) lies in part in the fact that the protection of the individual far outweighs the hazard of the vaccine. Vaccination at the same time removes its subjects from the reservoir of potential "carriers" of the virus which in former days was sufficient to make this disease one of the group of widespread immunizing infections of childhood. It may be doubted that this reason alone (if vaccination were of small benefit to the individual) would constitute a sufficient argument for the procedure. Pasteur's vaccination against rabies in the human is rendered feasible only by

reason of the circumstance that its administration can be restricted to the few in whom there is a high risk of rabies—those bitten by a rabid, or suspected rabid animal. The hazard of the vaccine in the population in general (postvaccination paralysis alone) would, numerically at least, far exceed the risk of the disease.

In respect to the employment of the principle of vaccination in poliomyelitis numerical considerations more like those of rabies than those in smallpox obtain. While a vaccination procedure might, as in smallpox, diminish the "virus reservoir" (conversion of non-immune individuals capable of multiplying and transmitting the virus to immunes not capable of doing so), if any prospective vaccine carried a hazard approaching the "natural" risk of disease in the individual, its applicability would be highly doubtful. Indeed, we have been through 1 experience where the risk of attempted immunization proved to be about as great as the risk of disease.[39a] If, as in the sense of selective immunization in rabies, the use of a poliomyelitis vaccination could be restricted to the few who are susceptible to *paralysis in poliomyelitis*, vaccination in poliomyelitis would be quite a different matter.

If this concept offers an explanation of one of the long puzzling paradoxes of the disease—a disease of warm weather but of cool climates—it at the same time introduces another—that the disease is one of cooler climates where the dissemination of the virus is less and not of warmer climates where it is more. This might seem almost to constitute an argument (seeing that the virus is preponderantly immunizing rather than paralyzing—and even more in warm climates and by reason of earlier exposure) that speeding-up the spread of virus rather than hampering it (isolation, quarantine, etc.) would be a more effective preventive measure against paralysis than any that we have. At any rate, harking back to Gorrie's attempt to prevent malaria (developing in hot weather) by chilling a room,* it does suggest that the principle of variolation (immunizing the young by inoculation of active virus in a hot room, as it were) should be explored as well as that of vaccination (attempts at further attenuation of the naturally highly "attenuated" virus) in the search for a better way of adding to the already relatively high protection against paralysis in natural infection with the virus.

Conclusions. Evidence derived from the age distribution of the recognized disease, from immunity as shown by the neutralization test and from actual virus detection indicates that the rapidity and extent of dissemination of the virus of poliomyelitis is the same as in the other "immunizing infections of childhood."

In respect to incidence of frank disease, age distribution, rapidity of development of immunity and extent of immunity, poliomyelitis exhibits the same differences between cooler and warmer climates as other "immunizing infections of childhood" in which subclinical immunization occurs.

The reason for relatively lower incidence of frank disease and higher subclinical immunization in warmer climates would appear to be the same in the group of subclinical immunizing infections of childhood and to lie in earlier exposure in warmer climates—at younger age when maternal passive immunity is still present in sufficient degree to protect against frank disease but to allow "modified" infection with resultant active immunity. The reason for earlier exposure in warmer climates would appear to lie in climatic and seasonal variation in the human host as a suitable "reservoir" for multiplication of the virus. There are indications that host-virus activity may tend to be seasonal in temperate climates and year-around in warmer climates.

* John Gorrie, a student of climate and fevers in Apalachicola, Florida, by way of perfecting his "technique" of fever prevention and cure, invented the gas compression process of refrigeration. There is no record that his treatment for malaria was successful—nor his invention—for him; but he is the father of air-conditioning, and all its application to health. Marshall Taylor's story of Gorrie (President's Address, Southern Medical Association in 1935[54]) is fascinating reading.

Studies relating to the control of infectious disease, the ultimate goal of which has been eradication, have long centered on the infectious agent and its mode of spread, not only as a *sine qua non* but as the *ne plus ultra* for prevention. The studies relating to subclinical immunization reviewed in this paper suggest that in poliomyelitis all the other elements entering into what constitutes an extraordinarily high degree of balance between this parasite and its host should be taken into consideration in attempts to prevent the one serious and exceptional sequel of infection with this virus—paralysis.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

UNDER THE CHARGE OF

JOHN E. GORDON, M.D.
PROFESSOR OF PREVENTIVE MEDICINE AND EPIDEMIOLOGY, HARVARD SCHOOL OF PUBLIC HEALTH
AND
W. LLOYD AYCOCK, M.D.
ASSOCIATE PROFESSOR OF PREVENTIVE MEDICINE AND HYGIENE, HARVARD MEDICAL SCHOOL
BOSTON, MASSACHUSETTS

THE STRATEGIC AND TACTICAL INFLUENCE OF DISEASE IN WORLD WAR II

BY JOHN E. GORDON, M.D. *
BOSTON, MASSACHUSETTS

(From the Department of Epidemiology, Harvard School of Public Health)

THE memory of men going ashore on a Normandy beach or approaching a Pacific island—American soldiers going into action—is still too vivid to leave any doubt as to where wars are finally won or lost. As certain as that may be, modern warfare is such that influences other than the courage of the men concerned or the quality of their leadership in combat, act strongly in determining the result. Disease and injury, and the quality and kind of medical care, constitute 1 such factor.

No attempt is made here to determine the relative importance of medicine and the other necessary services supplied to combat forces. It is likewise left to others—to the professional soldier, the statesman or the historian—to state the place of medical matters among activities that involve the whole of a population at war; the production of war materials, the management of economic stresses, and the maintenance of that fine balance of political and social interests within a nation and in respect to its allies. Few medical officers would attempt either evaluation; certainly not a civilian physician-soldier whose service was limited to the army in the field. The present analysis is confined to some of the ways that medical affairs enter into the management of military operations and the extent to which they influence the result. Since the experience and the data are wholly of army origin, the consideration of strategic and tactical influences of disease will relate only to that part of military activities. Most of the examples cited are from the European Theater of Operations of World War II.

RESPONSIBILITY FOR HEALTH. That the maintenance of the health of troops is a function of command is an accepted part of military theory[4] and so prescribed in the regulations of the United States Army.[26a] Time was when this meant principally an adequate attention to the care of the sick and wounded. The past half century has brought about a greatly broadened interpretation of this obligation, to the extent that the prevention of disease now ranks as a coördinate activity.

While modern military medical practice has 2 principal objectives, those of medical care and preventive medicine, the organization for provision of these services is by no means to be interpreted as consisting of 2 parts. These primary interests overlap, and the successful accomplishment of both objectives depends upon the extent to which they are integrated into a single practice, which recognizes no

* Formerly Chief of Preventive Medicine Division, Office of the Chief Surgeon, European Theater of Operations, United States Army.

sharp distinction between what is preventive and what is curative medicine.

Medical care in military practice has come to involve much more than the simple issue of death or recovery. Increasing attention is directed to the development of improved method for the prevention of physical defect after disease or injury, and for limitation of the period of disability.[25] Both features act toward the welfare of the individual, and from the viewpoint of military operations are important considerations as they affect available and effective manpower. They represent medical care directed toward preventive ends.

Preventive medicine has likewise developed far beyond its original interest in the control of the communicable diseases and a concern of environmental sanitation. The prevention of disease in general, whether it be communicable or non-communicable, is now an established part of the program for prevention,[21] together with an interest in the limitation of traumatic and other injuries. In military practice, clinical and preventive medicine have become so nearly equal in emphasis that medical influences in military operations might be approached equally well from a consideration of the strategic and tactical influence of health, as from the standpoint of disease.

Administrative Mechanisms in Military Medicine. Since the responsibility for health is a function of command, it follows logically that need exists for a command adequately informed of health activities and health methods. The medical officer and the other technical experts of the Medical Department charged with health matters through delegated responsibility, have equal need for an appreciation and understanding of military matters. The failure of a commander to understand and evaluate medical risks can be just as costly as to misjudge the fire power of the enemy. A lack of understanding on the part of the physician of that fine balance between military necessity and medical losses can be similarly inhibitive of the common effort which is the successful prosecution of a campaign. No remote suggestion is advanced in discount of the idealism that characterizes the practice of medicine; that is one of the finest parts of the profession, but war is not an idealistic business. It becomes necessary at times to look upon the health problems of a command from the standpoint of the group, to accept minor losses in order to effect major gains, and to weigh cost against military objective. The physician in military practice soon comes to appreciate that much of what he hopes to accomplish depends upon the support, the understanding and the aid that comes from his commanding officer. The medical officer must be equally cognizant of the objectives and requirements of command, for the measure of common accomplishment is generally determined by how well they understand each other.

These considerations are introduced because they are believed fundamental to an understanding of the influences that disease exerts on the conduct of war in the field, and to proper interpretation of the health record that results. This being the primary purpose of this presentation, rather than the methods and organization involved, a brief consideration of the 3 lines of approach to the management of health matters will suffice.

Command is called upon for a good deal more than intelligent understanding and direction of health activities. There is need for active participation. The good commanding officer gives interest and attention to the clothing, equipment and general well-being of his men, as well as to the adequacy of their training and the state of their military bearing. General Patton was the soldier, his primary concern with strategic and tactical matters. Perhaps that was the reason for his interest in the program of nutrition sponsored by the Medical Department, namely, that it fitted so logically into the framework of his strategic and tactical interests.

The direct responsibility for provision of medical care to the sick and wounded

falls wholly upon the Medical Department. The elaborate provision for casualties in the forward areas during the war just past has been thoroughly and adequately described,[27] from battalion aid stations, to collecting and clearing stations and to definitive care of non-transportable cases in field hospitals, or in sections of field hospitals to which auxiliary surgical teams were attached. The chain of evacuation extended to evacuation hospitals and back through the lines of communication to the great general hospitals.[28] Station hospitals cared for the needs of troops of the Communications Zone and of those of rest and reserve areas; and back of all were the major facilities of the Zone of the Interior for those seriously incapacitated or with disabilities of long duration.

A fundamental difference between the services directed toward medical care and those of preventive medicine is that successful accomplishment of the latter depends to a far greater extent on a coöperative effort of all arms and services. The supervision and control of preventive activities is, to be sure, a function of the Medical Department and much of the technical service is provided by that part of the army; the inspection and control of food supplies of animal origin by the Veterinary Corps; and the laboratory services so essential to the modern program of prevention. The unit surgeon is the health officer of his organization and he is aided by the special divisions of preventive medicine which are a part of all major commands. These strictly medical activities are however only a part of the comprehensive program for prevention. The Corps of Engineers has responsibility for the provision of potable water, the disposal of sewage and refuse and other activities which enter into the program for environmental sanitation. The food program of the army brings into play a close coöperation of Medical Department and Quartermaster Corps. The Medical Department is charged with the adequacy of the ration, the Quartermaster Corps provides it to troops and assures its quality. The design and provision of satisfactory clothing and equipment are other responsibilities of the Quartermaster which relate strongly to matters of health. The provost Marshal finds direct participation in health activities by way of the program for control of venereal disease, and the prevention of accidents. The Chaplain also has a concern with venereal disease control, and a still broader interest through matters of psychologic and moral well-being which enter so largely into the health of an army. The Special Services Corps was a principal agent in public health education. In summary, preventive medicine in the army as in civilian practice depends for its success upon a community effort. It succeeds in its objectives to the extent that the various arms and services are brought into a common program.

Classes of Military Casualties. Military casualties are divided into 3 categories, those of battle casualties, non-battle injuries and those the result of disease. Disease is thus set apart from injury as a source of disability, with a further distinction of injury as it relates to battle or non-combat origin. The separation of the many conditions involved is generally clear-cut, but for some the decision is made arbitrarily. Trench foot contracted by soldiers in the line is classed as a non-battle injury, although reasons of lesser moment have been advanced in claim of combat status. It is likewise evident that similar events are classified differently according to the circumstances under which injury took place. A gunshot wound of the hand incurred accidentally in a training area, or contracted anywhere as a self-inflicted wound, is a non-battle injury, and distinct from the battle casualty that results through contact with the enemy. The definition of terms that follow is from Army Regulations.[26b]

Patients are classified according to the primary cause of initial admission and re-

ported in 1 of 3 categories of cases: disease, injury, or battle casualty. In instances of patients suffering from both disease and injury at the time of initial admission, the most serious condition present is taken as the primary cause of initial admission and determines the classification. Patients admitted for a battle casualty and a disease or injury are classed as a battle casualty.

All cases other than those due to injury or battle casualty are classed as "disease." Included among the disease cases are patients suffering from reactions to medication other than acute poisoning, patients admitted for the sequela of an injury incurred prior to entering service, and p tients readmitted for the results of a traumatism (battle or non-battle) incurred during service.

The term "injury" includes traumatisms other than those defined as "battle casualty." The term "traumatism" refers to morbid conditions due to external causes. It includes acute poisoning, except food poisoning, the results of exposure to heat, cold and light as well as various types of wounds.

A battle casualty is a traumatism (wound or injury) which is incurred as a direct result of enemy action during combat or otherwise, or is sustained while immediately engaged in, going to, or returning from a combat mission. It does not include traumatisms occurring on purely training flights or missions. Psychiatric cases occurring in combat are not reported as battle casualties.

The measurement of losses from whatever cause is accomplished by computation of rates that relate to 3 principal demographic characteristics. The first of these, mortality, is the expression of the number of deaths from a particular cause that occur per unit of population and time, the ordinary unit of population being 1000 men and the interval of time 1 year. The rates for shorter periods are based on the assumption that the observed frequency would have continued over a year. The mortality rate represents a definite and certain military loss, irrespective of cause, of time or of nature, and is one of the absolute indices of the cost of war.

The morbidity rate expresses the number of persons affected by a given condition as determined by patients admitted to hospital or quarters, in relation to the same units of population and time as serve for mortality. Morbidity rates as so defined do not represent all persons affected but only those seriously enough involved to be absent from duty. Nevertheless, these indices of illness as employed in military practice are more satisfactorily indicative of the existing situation than is usual in public health or preventive medicine, because reporting is particularly good. The interpretation of the significance of any particular morbidity rate as an influence on tactical and strategical operations depends, in the first instance, on the duration of the disability ordinarily associated with the condition; secondly, on the expected fatality; and finally on the probability for complete recovery and return to duty

The commander of a military organization ordinarily finds the daily non-effective rate the most valuable single index of the health of troops. As the term indicates, this is representative of the number of men absent from duty by reason of disease or injury for each 1000 troop strength per day. The complementary value shows the proportion available for duty at any prescribed time.

Non-effectiveness in the European Theater. Experience of the European Theater of Operations in respect to non-effectiveness of troops by reason of medical disability is shown in Figure 1. It is to be noted here that these data and all others included in this study are from field records and therefore subject to revision and correction when the final analysis of individual case records is eventually completed. The provisional data are believed sufficiently reliable to establish relationships and trends. Some are from theater sources; a great part were made available by the Division of Medical Records, Office of The Surgeon-General, United States Army.

Discounting the early part of 1942 when the small troop strength accounted

for irregularities in the demonstrated pattern, each year of the 4 year period of World War II saw the high point of non-effectiveness centered about the early months of the calendar year and minimal values during the summer.[8] The seasonal incidence of upper respiratory infections was the dominating influence. Variations from year to year were not great until the latter part of 1944 when the values for all months increased precipitately over the established norm. This was coincident with the beginning of active operations in Continental Europe.

The division of this particular experience into the 3 components which make up the total non-effective rate (Fig. 2) gives ready demonstration of the factors involved. The non-effectiveness related to disease continued according to established pattern, with rates in 1945 almost identical with those that characterized 1944, the year just preceding the campaign. A significant part of the excess non-effectiveness came about through a greater frequency of non-battle injuries, principally trench foot. The most important variable was that of battle casualties, the data of Figure 2 demonstrating clearly that the high non-effectiveness of the campaign period was due to that cause.

The generalizations to be drawn from this experience are that year in and year out the principal cause of non-effectiveness of troops is disease. The losses from non-battle injuries are ordinarily much

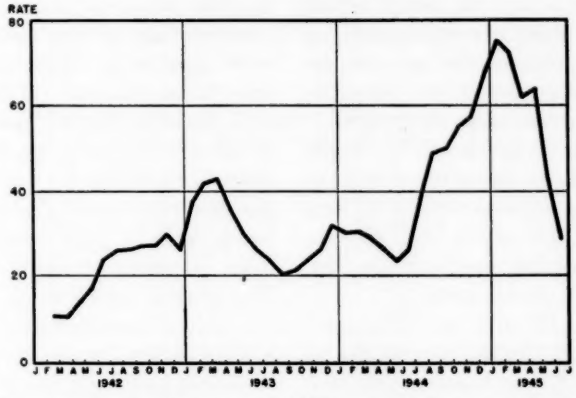

FIG. 1.—Average daily non-effective rates per 1000 strength, European Theater of Operations, U. S. Army, by months, February 1942 to June 1945 inclusive.

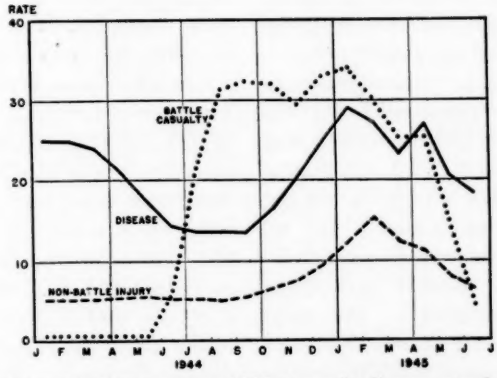

FIG. 2.—Average daily non-effective rates per 1000 strength, disease, non-battle injury and battle casualty, European Theater of Operations, U. S. Army, January 1944 to June 1945 inclusive.

less, about one-fifth those from disease. The non-effectiveness that comes from battle casualties is subject to great variation and is wholly related to the nature of operations. The impression is not to be left that the cost in battle casualties is unpredictable, for the expected losses in a major operation can be computed with an exactness rivalling that of disease and injury.

It is noteworthy that the rates for all 3 classes of casualties are susceptible to irregular fluctuations which can be related with much certainty to environmental, seasonal, or other ecologic factors.

The peaks of excess incidence that mark the behavior of battle casualties and nonbattle injuries are as outstanding as any introduced into the curve for disease through action of an epidemic in the common definition of the term.

Disease as a Factor in Military Operations. That disease as a cause of death and disability has become a matter of far less significance in the wars of the past half-century is a matter of general knowledge.[9] It is not so generally appreciated that the changes that have occurred are qualitative as well as quantitative.

The ratio of deaths from disease to deaths from battle casualties* for the wars of the 18th and 19th centuries was sometimes as great as 13 to 1. A generally accepted ratio was 4 to 1, as for example, in the Turko-Russian War of 1877–78[16] where deaths from disease numbered approximately 80,000 and those from battle casualties 20,000. The ratio during the campaign in the Crimea was even greater, with some 70,000 deaths from disease and 7500 from battle casualties among the French forces. Essentially two-thirds of the deaths that occurred in the Union Army during the American Civil War[24] were from disease, which marked an improvement over the Mexican War of 1846–47 when deaths from disease outnumbered those from battle casualties 7 to 1.[2] The record during the Spanish-American War[15] was less satisfactory, with an excess of deaths from disease over losses in battle in the proportion of about 13 to 1.

Fewer deaths from disease than from battle casualties were noted for the first time (Table 1) in the War of 1864 which Denmark waged against Austria and Prussia.[11] Both opponents established a ratio of 1 death from disease to 2 for casualties of battle.[12] The number of men engaged in that war was small, communications between the armies and home countries were good and environmental conditions were favorable; and yet this was a remarkable event, a turning point in the history of wars. The Franco-Prussian War of 1870–71 was the first major war[9] to see the new ratio maintained, by the German Army with a proportion of 0.86 deaths from disease for each battle casualty. The health record of the German Army has indeed been consistently good, for of 5 wars, dating from the Danish action of 1864 and including World War II, deaths from disease have been less than those from battle casualties with the single exception of the War of 1866 and that was close to parity. The Russo-Japanese War of 1904, the next great conflict after the Franco-Prussian War, gave the Japanese forces an opportunity to set a new record[20] of 0.37 deaths from disease per battle casualty. World War I was the first American experience in which a similar result was attained, providing troops in the active European campaign of 1918[18] be considered (Fig. 6). For the army as a whole and representative of all men under arms the ratio was still in favor of disease (Table 1). World War II brought a complete departure from previous experience, and a health record never approached previously in any war.

The gains which have been made in recent times in the cost of disease are primarily due to improved control of acute infectious processes. Not only are deaths far less frequent in proportion to those at risk, but the incidence of communicable

* Grateful acknowledgement is made to Lt. Col. Irvine H. Marshall, Medical Corps, United States Army, for the data of Table 1 upon which this discussion is based.

disease is decidedly less. This has brought significant changes in the qualitative character of the losses that still result from disease as distinguished from injury and battle casualties. Non-communicable disease has become a far more significant consideration. That the frequency of this class of disability had a direct relationship to military operations is illustrated by the data of Figure 3.

During the time that active combat operations involved only a small proportion of American troops in Europe, those of the Air Force based in Great Britain, the rates for neuropsychiatric disease were fairly stable and at a satisfactory level. For the whole experience, admissions for this cause constituted about 7% of all disease. The influence of major field operations is evident in the rise in frequency that took place in the summer of 1944 when the continent of Europe was invaded. The excess incidence was as sharply defined, and interjected as precipitously, as that of any epidemic of acute upper respiratory infection or other communi-

TABLE 1.—DEATHS FROM DISEASE AND DEATHS FROM BATTLE CASUALTIES IN THE PRINCIPAL WARS OF THE PAST 100 YEARS

War	Deaths		
	Disease	Battle	Disease : Battle
Mexican War, 1846–47 (United States)[1]	10,986	1,549	7.03:1
Crimean War, 1854–56 (French)[9]	70,000	7,500	9.33:1
Civil War, 1861–65 (Union Troops)[24]	186,216	76,216	2.44:1
Danish War, 1864 (German)[11]	310	738	0.42:1
(Danish)[12]	820	1,446	1.57:1
German War, 1866 (German)[17]	5,219	4,008	1.30:1
Franco-Prussian War, 1870–71 (German)[9,23]	14,904	17,225	0.86:1
Russo-Turkish War, 1877–78[16]	80,000	20,000	4.00:1
Sino-Japanese War, 1894–95 (Japanese)[9]	15,850	1,311	12.09:1
Spanish-American War, 1898 (United States)[15]	4,795	379	12.65:1
Philippine Insurrection, 1898–1902 (United States)[15]	4,409	1,036	4.26:1
Boer War, 1899–1901 (British)[3a,b]	11,377	6,425	1.77:1
War in South-West Africa, 1904–07 (German)[17]	689	802	0.86:1
Russo-Japanese War, 1904–05 (Japanese)[17]	21,802	58,257	0.37:1
(Russian, less Port Arthur)[9]	18,830	23,008	0.82:1
World War I, 1914–18 (French)[5]	1,750,000	924,700	1.89:1
(German)[6]	155,013	1,531,048	0.10:1
(United States, all troops)[23]	58,119	50,385	1.11:1
(United States, A.E.F. Europe)[23]	21,314	50,385	0.42:1
World War II, 1939–45 (United States, European Theater)[8]	1,432	122,384	0.012:1

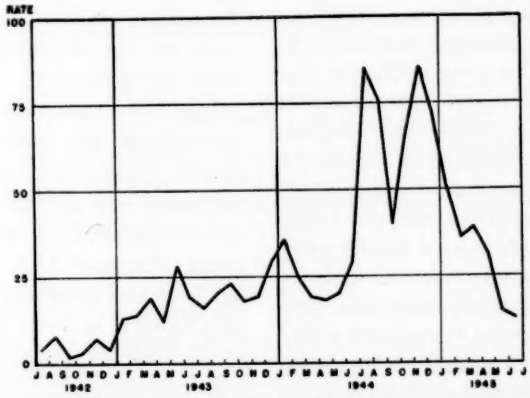

FIG. 3.—Neuropsychiatric conditions, admissions to hospitals and quarters, European Theater of Operations, U. S. Army, rates per 1000 strength *per annum*, by months, July 1942 to June 1945 inclusive.

cable disease. In July 1944, about one-fourth of medical admissions were of this class. As the pressure of assault operations was relieved, the frequency of neuropsychiatric conditions declined as promptly as it had arisen; despite the fact that this was a period of relatively active field operations marked by the rush across France and the approach to the German border. The outstanding difference was that this was a conquering army, with rapid and successful advance against an enemy that offered little resistance.

The frequency of neuropsychiatric disease again rose sharply when the attack was resumed in November; and a second peak of excess incidence duplicating that of the first was associated with the stubborn active defense action brought about by the Battle of the Bulge. Thereafter, the rates declined progressively and in marked degree, despite the active offensive action of February, March and April, when the Rhineland was invaded and the Inner Reich eventually occupied. The rates then were little more than during the relatively peaceful days in Great Britain before the continental campaign got under way.

The changing character of losses from disease is equally well illustrated by the kinds of conditions that entered into mortality rates from that cause in the European Theater. Considering the entire period of operations, 5 more deaths were recorded from alcohol poisoning than from all communicable processes combined, to include not only the usual epidemic diseases, but all other infectious processes such as tuberculosis and the pneumonias.

By either of the 2 principal criteria by which the effects of disease are judged, mortality and morbidity, the communicable diseases have decreased significantly as a factor of importance in military operations. Qualitatively the non-communicable processes have attained a significance out of proportion to previous experience. The effect that these changes have had on the total losses from disease are illustrated to advantage by an examination of the experience of the European Theater of Operations during World War II, as compared with the American Expeditionary Forces of World War I.

For each of the 4 years that the European Theater was in existence, disease was far and away the most frequent cause of admission to hospital or quarters (Fig. 4). The highest rate was in 1943, the widespread epidemic of influenza that occurred in the autumn of that year being the chief determining factor. The rates from year to year showed little variation, irrespective of whether the battle was fast or slow. A direct correlation between the activity of military operations and the frequency of disease was lacking. The numbers of persons affected were regularly great, since 1 out of 2 soldiers tended to suffer each year some disability from disease of sufficient degree to interfere with military duties. The regularly occurring annual peak of incidence in late autumn or early winter (Fig. 5) serves to demonstrate the overwhelming importance of acute upper respiratory infection as the dominant factor in morbidity for this class of disability.

No particular significance attaches to the experience of the first 2 years of the European Theater. The morbidity rates for disease were in all respects satisfactory and the health record good. The striking feature becomes evident in respect to the last 2 years. During the height of the campaign, which started in the middle of 1944 and ended in the early summer of 1945, the morbidity rates for disease as judged by admission to hospital and quarters was at a lower level (Fig. 5) than at any other time during the war. Proverbially and throughout the history of wars this is the time when losses have been great. Granted that many soldiers will not report sick during the height of military operations, and particularly in time of advance, nevertheless the fact that so few were seriously disabled by disease is perhaps the clearest evidence that could be advanced of the progress that has been

made in environmental sanitation and in the practice of preventive measures.

While disease ranked first among the 3 major categories of military casualties as a cause of disability, it was the least important as a cause of death. The absolute rate, 0.5 per 1000 strength per year, was inconsequential when compared with death rates for disease in other wars of this country, or indeed within the history of warfare.

strong second place in 1918 to a good third in World War II. Deaths from nonbattle injuries in the recent war exceeded deaths from disease in a greater proportion than deaths from battle casualties had exceeded disease in World War I. Comparing directly deaths from battle casualties and disease in the 2 wars, the ratio in the European phase of World War I was slightly better than 2 to 1; in the second war about 92 to 1.

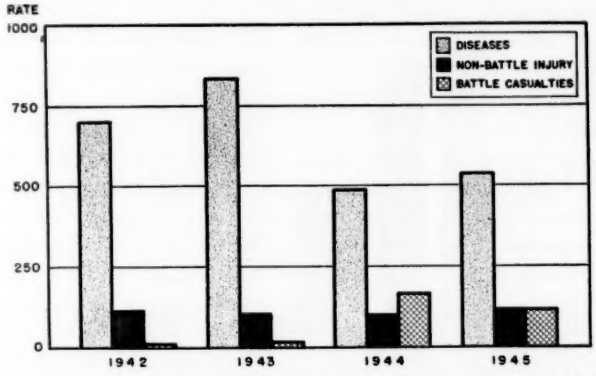

FIG. 4.—Admissions to hospitals and quarters, all causes, European Theater of Operations, U. S. Army, rates per 1000 strength *per annum*, February 1942 to June 1945 inclusive.

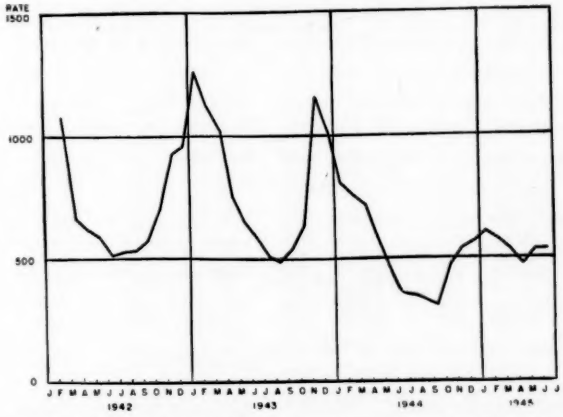

FIG. 5.—All diseases, admissions to hospitals and quarters, European Theater of Operations, U.S. Army, rates per 1000 strength *per annum*, by month February 1942 to June 1945 inclusive.

The 2 European Wars of the United States offer an opportunity for comparison of the changing trends in causes of death among military casualties (Fig. 6). Battle casualties were the principal cause in both instances, but disease dropped from a

Losses from battle casualties are difficult to compare because so much depends upon the type of campaign and the development of weapons of war. These 2 campaigns were fought in the same general region and against the same enemy; to

that extent the comparison is reasonable. At any rate, deaths from battle casualties were proportionately less in World War II than in World War I and it is reasonable to interpret that difference as related to improved methods of medical care. Expectedly the active open warfare of the more recent experience would lead to a greater proportion of casualties than the static trench warfare which characterized the greater hazards associated with a more highly mechanized warfare.

The experience thus far presented in respect to the casualties resulting from disease in World War II have related wholly to the European Theater of Operations. The experience for all 9 theaters of operation through June of 1945 is presented in Table 2, but carries no suggestion of a comparison of results or an

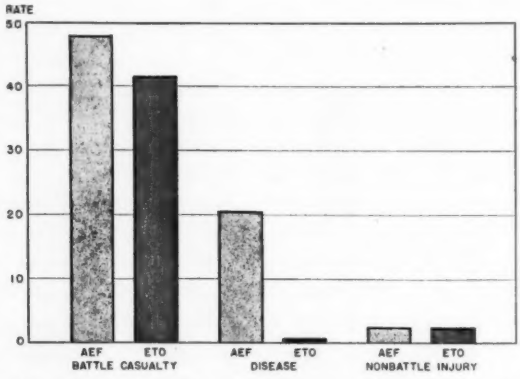

FIG. 6.—Causes of death in World Wars I and II, troops of the AEF (April 1917 to December 1918 inclusive) and of the European Theater of Operations (February 1947 to June 1945 inclusive), U. S. Army, average rates per 1000 strength *per annum*.

TABLE 2.—ALL DISEASES, ADMISSIONS TO HOSPITALS AND QUARTERS, TOTAL ARMY, CONTI NENTAL UNITED STATES AND THEATERS OF OPERATIONS, U. S. ARMY (CASES AND RATES PER 1000 STRENGTH PER ANNUM, BY YEARS, JANUARY 1942 TO JUNE 1945, INCLUSIVE.

Theater	Total		1942		1943		1944		1945	
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Total Army	14,120,214	667	2,047,606	671	5,163,760	768	4,550,658	606	2,358,190	609
Continental United States	8,578,265	653	1,699,134	699	3,793,588	739	2,261,800	564	823,743	571
Total Overseas	5,541,949	690	348,472	679	1,370,172	860	2,288,858	654	1,534,447	631
Africa-Middle East	123,336	946	7,783	1356	59,073	1107	42,468	896	14,012	587
China-Burma-India	308,806	929	5,951	1046	45,636	991	171,716	1077	85,503	707
Southwest Pacific	1,117,120	926	55,751	832	204,267	1046	465,289	840	391,813	1006
Mediterranean	1,148,934	849	9,618	451	406,619	943	558,051	846	174,646	726
South America	235,832	676	84,864	825	82,748	684	46,448	540	21,772	558
Pacific Ocean Area	626,954	600	72,812	494	239,851	813	221,457	561	92,834	448
Alaska	155,041	571	33,564	668	71,615	624	39,766	478	10,096	431
Europe	1,735,263	546	50,881	700	221,078	837	725,437	492	737,867	538
North America	90,663	534	27,248	672	39,285	548	18,226	433	5,904	382

Source: Medical Statistics Division, Office of The Surgeon-General, War Department, Washington, D. C.

so much of operations in 1918. While the differences in death rates from battle casualties during the 2 wars is not great, that in respect to disease is most striking. The losses in 1942–45 were but a fraction of those of the previous experience, essentially one-fortieth. Only in respect to non-battle injuries did the death rates of World War II rival those of World War I. The rates were almost identical despite attempted measurement of accomplishment in these several areas of military activity. It is wholly evident that losses from disease are subject to great variation according to differences in environment, the kinds of risk to which troops are subjected, the prevailing health hazards, and the prevalence of particular communicable diseases. It is, however, a matter of satisfaction that where environ-

mental conditions were similar and risks comparable, the morbidity rates for disease in overseas theaters compared favorably with those experienced by troops stationed in continental United States, which was 653 per 1000 strength *per annum*. Four theaters actually had better rates, although allowance must be made for the problems associated with recruits in the Zone of the Interior, and the greater resistance of seasoned troops who went overseas.

The North American Theater had the best morbidity rate for disease of all forms, 534 per 1000 strength per year. The European Theater, the largest in respect to troop strength, was next. The highest morbidity was experienced by troops of the Africa-Middle East Theater, followed closely by those stationed in the China-Burma-India area and in the South-West Pacific. The admittedly greater hazard of the Mediterranean area of Europe as contrasted with northwest Europe is reflected in average annual rates for disease of 849 and 546 per 1000 strength per year.

The analysis of the component causes which served to determine these rates, and a comparison of experience between theaters, is beyond the scope or intent of this presentation. The results universally obtained in respect to a particular disease long recognized as a peculiar hazard of war, are nevertheless so striking as to deserve special mention. In the European Theater of Operations where battle casualties were more numerous than in any other theater, both the mortality and the morbidity rates for tetanus were less than among troops stationed in continental United States, thousands of miles from a field of battle. The almost unbelievably good results, a single case and a single death during the whole period of operations in Europe, are attributable to the remarkable effectiveness of active immunization brought about by tetanus toxoid. No soldier left America without immunization and the greater rates in the Zone of the Interior, of themselves inconsequential, were related to tetanus infection among recruits before immunization had been accomplished. The detailed circumstances of this experience in all theaters of operation have been presented by Long and Sartwell.[14]

World War II involved more men and extended over a greater geographical area than any other in history. The successful result that accrued to the American arms was influenced in forceful degree by the action of favorable casualty rates for disease previously without precedent. The overall results speak for themselves. The extent to which disease influenced individual campaigns remains to be examined, first in respect to long-term strategic action and secondly as an immediate tactical influence.

Strategic Influence of Disease. Rarely does the practice of epidemiology on the grand scale reach such potentialities as in war. The epidemic of typhus fever that eventually involved American troops in Germany in 1945 constitutes an outstanding example of the strategic influence of disease in field operations. The epidemic was evident well in advance of the time that it became an immediate military problem, with the result that plans and an organization for meeting the situation got under way as early as 1941.

The probable situation at different times in the projected course of military operations was analyzed in detail. Arrangements for a cordon sanitaire along the Rhine River with tentative ports of entry were set up 3 years before an American soldier crossed that river. A significant change in procedure was incorporated in the plan in 1944 when the value of the newly developed insecticide DDT became evident. Unit responsibility for control measures in the particular area of influence was recognized as the fundamental approach. Provision was made at theater and army echelons for teams trained in diagnostic and insect control procedures to furnish the necessary aid and consultation to unit surgeons. The proper administration of the cordon

sanitaire was made the responsibility of the army group.

Typhus was found in Germany in March 1945 as anticipated, in all a total of 17,000 cases.[1] The number of secondary infections was surprisingly few. The outbreak was eliminated within 3 months. During this time only 3 American soldiers contracted the disease, 2 of them physicians engaged in typhus control. The results contrast with what took place in 1918, although at that time material progress had already been made in knowledge of this epidemic disease and in measures for its control. More than 5,000,000 persons[13] had typhus fever in Russia alone[7] and deaths have been estimated at 2,000,000; in Serbia[10] essentially one-fifth of the population was involved in the epidemic, with 150,000 deaths[22] in a 6 month period of 1915. The potentialities for a similar major epidemic in 1945 were decidedly great, for the existing circumstances were not dissimilar from those of 1918. The difference in result was determined by the advances in technical knowledge that had taken place within recent years, and the new measures for control that were provided. It depended upon careful planning at staff levels, adequate provision of supplies, organization of the special United States of America Typhus Commission, and finally the thorough mobilization of the field medical services who did the work of control. From a strategical standpoint, the casualties from this cause among military personnel were negligible. Not a single death occurred from a disease recognized as one of the great pestilences of man, and characterized by a notably high fatality. Still more important was the absence of any interference with military operations at a critical time in the developing final offensive. The army suffered no significant losses itself, nor was it called upon because of typhus to provide medical and other care to an overwhelmed civilian population of occupied territory.

Malaria in the Southwest Pacific constituted a strategic problem of equal importance to that of typhus fever in Europe. Advance planning had likewise been concerned with the provision of malaria control supplies, the development of method, and the training of personnel to combat this communicable disease. The early years of operations in that theater were attended with shipping difficulties. It is a function of theater authority to allot tonnage and a choice had to be made among the various classes of supplies assembled at ports of debarkation. The choice was made with the result that troops taking part in the early campaigns in the Solomons and in New Guinea were without sufficient malaria control supplies and lacked specially trained control organizations. The casualties from malaria were high. In the South Pacific Area the attack rate for malaria reached 696 per 1000 per year in August 1943. In the Southwest Pacific Theater the attack rate early that year exceeded 400. Four American and 2 Australian Divisions were incapacitated for periods of more than 6 months. At one time more than 30% of available beds in the Southwest Pacific Theater were occupied by malarial patients. Subsequently the situation improved greatly. Survey and control units were assigned to field control, the necessary supplies were shipped and strong emphasis was placed by command on the improvement of malaria discipline among troops. The morbidity rate for malaria declined steadily during the latter half of 1943, and in 1944, so that by 1945 the attack rate was less than 40 cases per 1000 troop strength per year in the better areas. The extent of the problem is indicated by the loss of 800,000 man days from malaria in the Southwest Pacific Theater in 1943, this constituting a fourth of the losses from all disease. The proportion was reduced to 5% in 1944; and the old ratio of greater losses from disease than from battle casualties again returned to the more favorable circumstance which characterizes modern warfare. The strategic

problem was met, but after avoidable losses.

The principle in facing a problem where the influence of disease is involved, is a question of how many effectives can be maintained in the line. Medical weapons and medical supplies may be as important a consideration as the number of troops made available or the basic military supplies of ammunition, rations and motor fuel.

little variation (Fig. 7). The rate for the year 1944 was actually the best of the 4 years despite the autumn campaign in France and appreciable losses in the last 2 months of that year from trench foot, a major item in the category of non-battle injury. The excess rate for 1945 was almost wholly of that origin and yet the rate for the year was not greatly in excess of the first training year of 1942.

The attack rate for disease was much

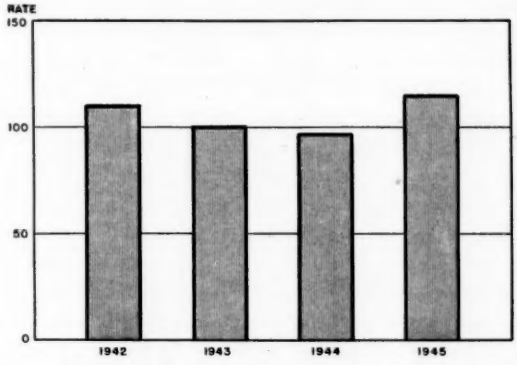

FIG. 7.—Non-battle injuries, admissions to hospitals and quarters, European Theater of Operations, U. S. Army, rates per 1000 strength *per annum*, February 1942 to June 1945 inclusive.

TABLE 3.—NON-BATTLE INJURIES, ADMISSIONS TO HOSPITALS AND QUARTERS, TOTAL ARMY, CONTINENTAL UNITED STATES, AND THEATERS OF OPERATIONS, U. S. ARMY (CASES AND RATES PER 1000 STRENGTH, PER ANNUM, BY YEARS, JANUARY 1942 TO JUNE 1945, INCLUSIVE)

Theater	Total		1942		1943		1944		1945	
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Total Army	1,921,342	91	294,288	96	624,849	93	666,209	89	335,996	87
Continental United States	987,163	75	230,366	91	412,655	80	270,536	67	73,606	51
Total Overseas	934,179	116	63,922	125	212,194	133	395,673	113	262,390	108
Alaska	41,341	152	7,619	152	20,852	182	10,558	127	2,312	99
Southwest Pacific	166,758	138	11,963	178	33,317	171	77,046	139	44,432	114
Mediterranean	179,581	133	2,040	96	64,075	149	91,063	138	22,403	93
North America	21,160	125	6,330	156	9,747	136	4,065	96	1,018	66
Africa-Middle East	14,679	113	928	162	7,469	140	4,712	99	1,570	66
Pacific Ocean Area	111,366	107	15,379	104	33,590	114	43,648	111	18,749	90
European	335,445	105	8,023	110	26,497	100	143,201	97	157,724	115
China-Burma-India	31,541	95	460	81	3,893	84	15,385	96	11,803	98
South America	32,308	93	11,180	109	12,754	105	5,995	70	2,379	61

Source: Medical Statistics Division, Office of The Surgeon-General, War Department, Washington, D. C.

NON-BATTLE INJURY AS A STRATEGIC AND TACTICAL INFLUENCE. The experience of the European Theater of Operations may be taken in illustration of the regularity with which losses from this class of military casualty occur. Irrespective of whether operations relate to a time of training or to an active campaign in the field, the number of men lost by reason of non-battle injury is subject to greater than that for non-battle injury, varying from a ratio of 8 to 1 in the most unfavorable year to a relationship of 5 to 1 under the most favorable conditions (Fig. 4). The difference between the 2 conditions is less marked when judged on the basis of the resulting non-effectiveness, since the period of disability from injury is longer than that for disease (Fig. 2).

A comparison of morbidity rates for non-battle injury in the several theaters of operation shows a variation in experience considerably less marked than for disease (Table 3). Without exception, casualties from non-battle injury were more frequent in theaters of operation than in continental United States, since no theater approached the average rate of 75 per 1000 strength per year established by troops of the Zone of the Interior. The influence of environment on the record attained was strongly evident. The Alaskan Theater with 1 of the best rates for disease had the highest rate for non-battle injuries, and conversely the China-Burma-India Theater, with a rate of 929 per 1000 per year for disease, had the admirable record of 95 for non-battle injuries. Again it is to be emphasized that no direct comparison between theaters can be made on the basis of these crude rates because environmental and tactical conditions varied greatly. The important consideration is that these are in large part preventable conditions, and the rate under a given set of conditions can be influenced by the extent to which preventive measures are applied. The important component conditions of motor accidents and trench foot are decidedly in point.

Trench foot in Attu, in Cassino and in the European Theater was a strategic problem of preventive medicine. The condition is an accompaniment of campaigns in wet cold climates. In a given week, in November 1944, 3000 cases occurred in a single field army. In the months that followed, all combat troops of the European Theater were involved in varying degree.[8] Field control was strictly an epidemiologic problem. Attack rates were shown to bear a definite relationship to the existing tactical situation. The risk varied as troops were on the offense, engaged in holding actions, under static conditions, or in rest areas. The kind of weather exerted an influence, likewise the character and amount of clothing and equipment. Qualities of the terrain could be demonstrated as a contributing factor and of decided significance were the

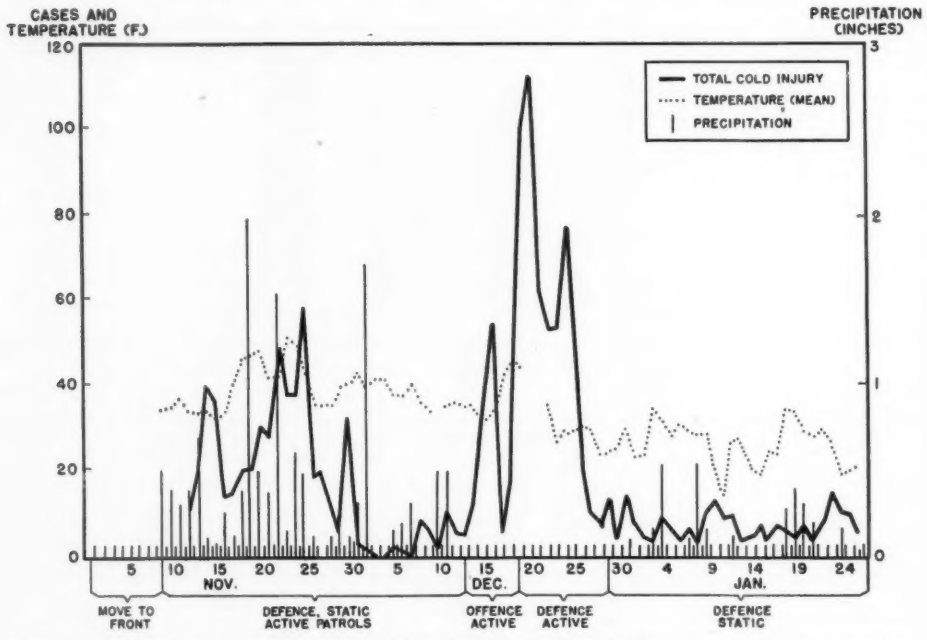

FIG. 8.—Cold injury in an infantry division, frequency of cases by type of military operation, temperature (° F.) and precipitation in inches.

methods of management of troops. The incidence was by no means uniform. Certain armies, divisions, regiments and even battalions suffered inordinately in comparison with neighboring units operating under similar environmental circumstances. Riflemen were affected beyond all other occupational army groups, with more than 90% of cases in this important component of the ground forces. The importance of this circumstance becomes evident when translated into terms of effective combat strength. A loss of 15,000 men from trench foot is seemingly the equivalent of 1 division and yet with 4000 riflemen to a division and 90% of the casualties within that group, the actual loss is 4 effective fighting divisions. The contributing causes of the condition and its incidence varied from place to place and from time to time. The principle of prevention is evident—an epidemiologic analysis of cause and effect (Fig. 8) and a fitting of control measures to the individual circumstances.

Three times in the course of World War II, trench foot was encountered as a strategic problem and 3 times the losses were beyond calculated risk or reasonable expectancy. The invasion of the Japanese mainland would have presented a fourth situation, for the environmental conditions predisposed in a degree comparable to those of Italy and northwest Europe. The combined opinion and effort of several arms and services went into the development and initiation of a plan which included the chief considerations of clothing and supplies, education of the individual soldier, the organization of special trench foot control units, and the administrative action of command. What was done never came to test, for hostilities ended before Japan was invaded. It behooves any future planning group concerned with a campaign in wet cold climates to review that plan.

Summary. The strategic influences of disease on military operations are understood to include those medical problems continuously present and existing by reason of environmental or ecologic influences peculiar to a given situation. They may represent problems of disease, as with malaria; or of injury, as indicated by the continually existing accident problem. Conversely, strategic problems may be temporary and unusual, but not unexpected. They are the carefully defined results of an anticipated change in ecologic equilibrium. When American soldiers set out for the Normandy beaches in June 1944, difficulties in respect to trench foot were certain to occur the following autumn and winter, with the extent of losses dependent upon the adequacy of plans and the sufficiency of supplies for prevention and control. The counterpart in respect to disease is illustrated by typhus fever in Europe, existing under conditions incident to war, although a disease normally absent.

Medical problems of tactical significance are those relating to the immediate situation. They may be so far-reaching as to involve most of the members of a military community. More commonly they are localized and sometimes highly individual. Under all circumstances they require prompt analysis of the situation and the institution of specifically directed control measures. The suddenly developing epidemic of influenza in Great Britain in 1943 was an example of a widespread tactical problem in military preventive medicine. The more usual illustration was that of an excess accident rate in a Quartermaster depot, the abnormal prevalence of venereal disease at a port of embarkation, or the series of peculiar illnesses at a chemical warfare service impregnation plant. These are the problems of the day and find complete analogy in similar occurrences of civilian communities.

The utilization of the medical services of a combat force to meet the strategic and tactical problems introduced into military operations by reason of disease requires more than adequate care of the sick and wounded. Many of the more important problems are essentially mat-

ters of preventive medicine. They are of 2 kinds: the group problems of the military community and the health problems that relate to the individual soldier. The group problems extend much farther than the control of epidemics and the management of communicable disease. Under modern conditions the infections become progressively less important and concomitantly non-communicable processes attain increasing significance—injury, accidents, trench foot—and a variety of nutritional, neuropsychiatric and other diseases, unrelated to communicability and yet of epidemiologic importance.

The health problems of the individual soldier rate equal importance with strictly group problems, since clothing, housing, nutrition and psychologic management act importantly in decreasing the slow attrition from disease and disability, which if exaggerated can approach in losses those that result from epidemic disease.

A basic principle in minimizing the adverse strategic and tactical influences of disease on military operations rests in a protective maintenance applied to men as well as to machine. And that is preventive medicine in its simplest terms.

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PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

G. LYMAN DUFF, M.A., M.D., PH.D., F.R.S.C.
STRATHCONA PROFESSOR OF PATHOLOGY, MC GILL UNIVERSITY

AND

E. G. D. MURRAY, O.B.E., M.A., L.M.S.S.A., F.R.S.C.
PROFESSOR OF BACTERIOLOGY AND IMMUNITY, MC GILL UNIVERSITY
MONTREAL, CANADA

ORAL IMMUNIZATION

BY C. E. DOLMAN, M.B., B.S., M.R.C.P., D.P.H., PH.D. (LOND.), F.R.S.C.
PROFESSOR OF BACTERIOLOGY AND PREVENTIVE MEDICINE, THE UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER, CANADA

RESEARCH MEMBER, CONNAUGHT MENIBAL RESEARCH LABORATORIES UNIVERSIUY OF TORONTO

MANKIND seems to enjoy placing a wide variety of objects into the mouth, but displays a widespread dislike of the needle puncture. From these impulsions, which are perhaps primordial, may derive much of the preference likely to be shown for oral ingestion rather than hypodermic injection of specific therapeutic and prophylactic substances, whether narcotics, endocrines, vitamins, sulfonamides, antibiotics or immunizing agents.

Sounder reasons for this preference may, of course, be advanced. For instance, peroral administration is simpler and cheaper, involving neither apparatus nor professional attendants. The obviation of local reactions at the injected site is also important, especially when dealing with sick or enfeebled patients, with subjects allergic to some bacterial product, or whenever it is essential that a minimum of hours should be lost from work, *e. g.*, by doctors, nurses and persons in the armed forces. Again, the possibility of a quicker rate of immunity development embodies many obvious advantages. Enthusiasm among immunologists for oral vaccination has been discontinuous and scattered, but understanding of its limitations has been only tentative. Hence, even the most hardened sceptic may experience at times a powerful attraction to the faith that an effective multiple antigen might be devised which could be swallowed by anyone whenever the occasion demanded; while a few of the more optimistic appear unable to exorcise themselves from a vision of some omnivalent pill, to be put out in a bottle discrete in size, elegant in shape, and of various styles to match the bedroom dressing-table décor of all smart people! These considerations, bolstered by the undeniable evidence that certain microörganisms or their toxins customarily gain access to the human body through the gastro-intestinal tract, perhaps account for the recurrent interest shown in oral immunization by reputable investigators. They no doubt also serve as inspiration for the unflagging eagerness of some manufacturers to feed the gullible with specious half-truths and impotent preparations.

Counterbalancing the possible advantages of the oral route are certain *a priori* handicaps to its effective use. The vagaries of human nature could obviously make the very convenience of this method an inducement for taking the medicine only as the spirit moved, rather than according to prescription; and the community, no less than the individual, would suffer the consequences of such sporadic and unrecorded self-administration. The already high incidence of unfulfilled obligations to seek specific protection against infection in the interests of one's own and one's neighbor's health might climb rather than fall; while the present meager statistics on the immunity status of given populations would be

further vitiated. Again, licensing authorities face imposing difficulties in attempting to check the composition and antigenic properties of a vaccine to be administered in pill, capsule or lozenge form, or of an elixir alleged to contain some bacterial metabolite of extraordinarily subtle virtue. In fact, it is no prejudgment of the issue to state that advocacy of oral immunization tempts the streak of charlatanism present in too many of us.

The Plan of Approach. However, the main purposes of this review are not to discuss the pros and cons of immunization *per os* in terms of psychologic and commercial problems involved, but rather to assess the feasibility and efficiency of the method as judged by evidence presented in the extensive and often conflicting literature; and also, to attempt a brief elucidation, in terms of modern immunologic conceptions, of the factors contributing to whatever theoretical and practical inadequacies may become apparent. The topic will not be reviewed from a narrowly historical or evolutionary standpoint; nor can reference be made to every publication bearing upon it. In the earlier years, a majority of reports on oral immunization appeared in the foreign journals, chiefly French and German. In some instances, these journals have not been available; in others, records of results which seemed unduly repetitive or trivial have been omitted. Some important work may have been unintentionally overlooked; but an effort has been made to accord fair credit and representation to the more significant contributions, and readers desiring to supplement the biblography can readily do so by consulting thesereferences.

From the beginning, investigators have tended to acquire interest in oral immunization out of concern with some human or animal infection for which the alimentary canal was believed the customary portal of entry, or the main seat of injury. Pasteur, for instance, made the earliest clear-cut reference to the possibility of immunization by ingestion, in a footnote to a paper on the etiology of anthrax, presented in 1880 to the Academy of Sciences in Paris, in collaboration with his colleagues Chamberland and Roux:[123] "Chickens that have been given food infected with the microbe of fowl cholera, and do not die, may have been vaccinated. Hence one may ask if one could not manage to vaccinate sheep against anthrax by submitting them previouslyand gradually to meals infected with the spores of the parasite."

A quarter of a century later, in 1906, Calmette and Guérin,[28] having become convinced that tuberculosis of man and animals, even in its pulmonary manifestations, was of intestinal origin, reported that they had thence been led to investigate "if it would be possible to vaccinate animals susceptible to tuberculosis against the natural infection by causing them to absorb through the digestive tract, young, while modified, attenuated or avirulent tubercle bacilli."

Again, Besredka's theory of local immunization largely stemmed from experiments on the problem of infecting mice orally with paratyphoid bacilli. The crucial observation was recorded thus in a paper by himself and Bassèches:[18] "One day, lacking fresh mice for a control experiment, we thought of using a mouse which had received, 1 month previously, paratyphoid bacilli by mouth. Having injected this mouse with a certainly fatal dose of a virulent paratyphoid culture, what was our surprise to find it, next day, gaily chewing its oats as if it had received nothing."

These examples may serve to illustrate the point that there has been all along a disposition to assume that those microorganisms and their toxic metabolites which cause disease by naturally traversing the alimentary tract of man or animals, or which seem to exert their major pathogenic effects thereon, are the ones most likely to prove antigenic when taken by mouth. The validity of this assumption need not be discussed, still less accepted at this stage. But its provisional adop-

tion suggests a convenient order to follow in the main part of this review.

Classification of Pathogenic Agents Under Review. Consideration will first be given to those pathogenic agents to which the term "enterotropic" may, with some reservations, be applied. Succeeding sections will review these agents in turn, arranged roughly according to diminishing degrees of apparent natural affinity for the intestinal tract. Since oral immunization is a process directed against microörganisms or their toxins, rather than against clinical syndromes, the sequence to be followed will be indicated here by listing representatives of each section.

1. *Eberthella typhosa, Salmonella paratyphi B* and other members of the Salmonella group.
2. *Shigella dysenteriæ* (*shigæ*), and other members of the Shigella group.
3. *Mycobacterium tuberculosis.*
4. *Clostridium botulinum toxin,* and *Staphylococcus enterotoxin.*
5. *Pasteurella pestis, Brucella abortus,* and certain animal pathogens.
6. *Staphylococcus, Streptococcus, and pneumococcus*
7. *Miscellaneous preparations, including mixed "cold" vaccines.*

Each of these agents will be scrutinized in the light of the relevant reports. Critical comments will only be interjected where these seem necessary for proper interpretation of results. Criticisms of a general nature will mostly be reserved for the final discussion.

Eberthella-Salmonella Organisms. More widespread use of antityphoid inoculations has been delayed by disputes about the significance of the various humoral antibodies which may be detectable after vaccination, by failure to recognize that immunity is in any event relative rather than absolute, and by the argument that improved standards of community sanitation and personal hygiene have been of paramount importance in reducing the incidence of typhoid fever in recent decades. Faulty methods of manufacture, and the administration of inadequate dosages of typhoid vaccine, have also led to disappointment. Nevertheless, few authorities now remain unconvinced of the value of antityphoid inoculations—particularly in time of war and other emergencies, or in areas where sanitary standards are low. A good summary of the earlier statistical data bearing on the efficacy of this practice has been provided by Harvey.[87]

The reactions liable to follow subcutaneous injection of the vaccine are admittedly a serious handicap. Indeed, the recurrent efforts made to provoke effective immunity by some form of peroral vaccination largely stem from a desire to avoid these untoward reactions. The results obtained by earlier workers were on the whole so disappointing that interest in this mode of administration lapsed, until revived by Besredka's vigorous championship of the local immunity concept. He visualized oral vaccination against the enteric group of infections as the most important and logical application of his principles. His contentions will later be critically appraised.

Typhoid fever is now recognized as one of a large group of related infections. The similarities in epidemiologic and immunologic characteristics between typhoid and paratyphoid A and B fevers led to the custom of adding *S. paratyphosa A and B* to the suspension of *Eb. typhosa* in the preparation of the mixture known as T.A.B. vaccine. More recently, these 3 microörganisms have been assigned a place in the still-enlarging Salmonella group, where they are linked, under the Kauffmann-White schema of classification, with some 150 other known types of human or animal pathogens, possessing certain cultural, biochemical and agglutinogenic properties in common. The many reports of earlier workers on oral immunization against typhoid and paratyphoid fevers will therefore be considered together with the few reports involving such related types as *S. typhi murium.*

In general, reports on oral immunization against enteric infections have furnished evidence of the following types: (*i*) Levels

of titratable antibodies attained in experimental animals or man following vaccination.

(*ii*) Protection displayed by vaccinated laboratory animals or man against the homologous pathogenic agent.

(*iii*) Statistical data on specific resistance to infection following vaccination of population groups in endemic or epidemic areas.

The first 2 of these headings may conveniently be merged for present purposes.

Effect of Vaccination per Os on Specific Antibody Formation and Immunity Development. In 1904, Wright[186] reported administered heat-killed cultures of *Eb. typhosa* to 7 persons by mouth, 3 of whom developed a marked increase in the bactericidal power of their blood, while 4 showed a decrease. One person complained of diarrhea and constitutional disturbances, but the others suffered no reaction. In the same year, Tchitchkine[169] fed live typhoid cultures to rabbits, and noted the appearance of agglutinins, complement fixing bodies, and precipitins in the blood serum. Sera from such animals gave no passive protection. Ingested typhoid culture filtrates sometimes evoked weak agglutinin production in rabbits. Loeffler[108] reported in 1906 having conferred some measure of immunity upon white mice and field mice, which had been fed repeatedly with heat-killed cultures of *S. typhi murium*. Some of these mice proved resistant to virulent, living cultures given orally, but not to similar cultures injected subcutaneously or intraperitoneally. Since the orally vaccinated field mice showed no agglutinin formation, Loeffler concluded that their acquired immunity was not general, but localized in the intestinal mucosa. Two years later, Wolf[184] obtained essentially similar results with white mice, which developed some resistance to ingestion of virulent cultures of *S. paratyphi B*, after prolonged feeding with broth cultures of living but avirulent strains. He noted that specific bacteriolysins developed, but no agglutinins.

In 1910, Leishman[104] reported discouraging results in man from attempts at peroral immunization with typhoid vaccine. His colleague, Major Harrison, had "endeavored to guide dead typhoid bacteria past the dangers of the stomach into the intestine," by administering to fellow officers in the British Army, pills containing typhoid bacilli suspended in a matrix of fat and stearin. Others were given the bacilli suspended in lard, in gelatin capsules. Some subjects had gastric disturbances, and the effects upon the bactericidal power of the blood were irregular.

Other workers of this period reported somewhat more promising results with laboratory animals. Kutscher and Meinicke,[100] for instance, in 1906 claimed to have immunized guinea pigs against *S. paratyphi B* and *S. enteritidis* by feeding a single large meal of parsnip pulp mixed with a 24 hour broth culture of either one of these microörganisms. Four weeks after the meal, most of these animals were resistant to 1000 to 10,000 minimal lethal doses injected intraperitoneally. Similar experiments with typhoid bacilli were unsuccessful. These workers also fed young calves, sheep, goats, dogs and a horse with 1 large dose of paratyphoid culture. No agglutinins developed. Again Hida and Toyoda[90] gave guinea pigs by mouth capsules containing typhoid, dysentery or cholera bacilli predigested with trypsin and pepsin, and noted development of serum agglutinins and bacteriolysins. Ingestion of heat-killed cultures provoked no antibody formation. Shiga,[164] on the other hand, demonstrated some immunity development in rabbits given heat-killed paratyphoid cultures by mouth. Moreover, Brückner[22] reported that mice given several daily ingestions of an avirulent culture of *S. paratyphi B* subsequently withstood a normally lethal subcutaneous dose of virulent culture. Disagreeing with Loeffler, he contended that intensive vaccination *per os* could give rise to a true general immunity.

In 1911, Metchnikoff and Besredka,[113] having shown that typhoid fever could be transmitted to chimpanzees, attempted to

vaccinate 1 of these anthropoids by giving orally on 2 occasions, 15 cc. of heat-killed *Eb. typhosa* culture. When challenged 11 days later with a large dose of living typhoid bacilli, sufficient to cause severe illness in a control ape, the vaccinated animal developed only a mild pyrexia. However, an attempt to reproduce this result was unsuccessful. Simultaneously, Courmont and Rochaix[45] vaccinated goats and rabbits orally and rectally, the latter route being better tolerated. Three doses of a polyvalent suspension of 8 day, heat-killed cultures of *Eb. typhosa* were administered rectally, at intervals of a few days, each dose being 100 cc. for rabbits, and 250 cc. for goats. Agglutinating, bacteriolytic and bactericidal properties soon appeared in the serum, but the titer of agglutinins did not rise above 1:50. Fifteen days after the last rectal instillation of vaccine, rabbits were able to resist, almost without symptom, an intravenous injection of virulent *Eb. typhosa* which killed controls in 28 hours. An unstated number of human subjects also received 3 doses of 100 cc. of vaccine per rectum, at 5 day intervals. There were no unfavorable reactions, and circulating antibodies appeared after the 10th day. Courmont and Rochaix[46] soon afterwards observed that rabbits vaccinated rectally resisted typhoid "toxin," comprising an 8 day culture of *Eb. typhosa* heated to 53° C., in a dosage which killed normal rabbits within a few hours. The serum of vaccinated animals neutralized the "toxin" when mixed therewith.

In 1914, Lumière and Chevrotier[109] reported experiments with a powdered form of polyvalent vaccine, prepared from a mixture of washed, centrifuged and dried typhoid, paratyphoid, and coliform bacilli. The product, termed "entéro-vaccin," was administered in keratin capsules to guinea pigs, rabbits and humans, without any resulting fever, diarrhea, or other reactions of the types encountered by Courmont and Rochaix. Four months after receiving by mouth a total dosage of 3 billion bacilli per kg., taken in 3 portions at 8 day intervals, guinea pigs and rabbits showed a definite and lasting immunity against a normally fatal intravenous injection of any of these organisms. The enterovaccine of Lumière and Chevrotier was soon submitted to trial by Dubarry[61] on a large number of war prisoners, none of whom complained of any ill-effects from the pills. In 1923, in a valuable review of vaccination by the buccal route, Calmette[24] stated that many physicians in France had used enterovaccines, and that in a number of cases circulating agglutinins had developed.

Besredka's Animal Experiments on Oral Immunization. In 1918, Besredka and Bassèches[18] reported that mice acquired a specific immunity from paratyphoid bacilli given by mouth. This immunity developed slowly, and was slight and transitory unless the mice ingested living cultures. The titratable antibodies were not mentioned. This paper, and another published by Besredka[11] soon afterward, initiated a series of publications which greatly influenced conceptions of immunity, particularly as regards oral immunization against enteric infections.

Besredka noted that rabbits became more susceptible to living paratyphoid cultures given by mouth or intravenously after previous ingestion of ox-bile. He asserted that the bile caused desquamation of the intestinal mucosa, thus facilitating absorption of the bacilli; and he deduced that the natural immunity of rabbits to orally administered paratyphoid bacilli was localized in that mucosa. Moreover, although a normal rabbit gained no protection against paratyphoid bacilli from swallowing killed cultures, it became "parfaitement vacciné" after previous sensitization with bile, and was then refractory to bacilli introduced by mouth or intravenously. Since this acquired immunity was established in 3 to 4 days, Besredka held that participation of antibodies in its mechanism was excluded. It in fact also be localized.

Besredka soon extended his conceptions to cover problems of susceptibility and

resistance of the rabbit to experimental infection with Shiga dysentery bacilli,[12,14] and typhoid bacilli.[13,15] He now urged oral vaccination for human groups exposed to typhoid and paratyphoid fevers and to bacillary dysentery; arguing that a maximum defensive response should be evoked in the intestinal mucous membrane when microbes reached there directly. Thereafter, a formidable body of doctrine, and a queerly confused terminology, burgeoned on shallow-rooted experimental data, until by 1927 the theory of local immunization had been extended by Besredka[17] to embrace many types of infections besides those having the intestinal tract as customary portal of entry. These claims were soon disputed. Zingher and Soletsky[190] vaccinated bile-prepared rabbits orally with living or dead paratyphoid bacilli, and produced neither agglutinin nor resistance to similar bacilli injected intravenously. (Besredka[16] had noted that circulating agglutinin titers up to 1:80,000 might be attained in 3 or 4 weeks by rabbits sensitized with bile, and then given a sublethal dose of living paratyphoid bacilli by mouth. These agglutinins had quickly diminished, and did not reappear after subsequent ingestions of culture—an observation which he interpreted as additional proof of the intestinal wall's impermeability in orally vaccinated rabbits, and also of the irrelevance of humoral antibodies.) The only experimental findings of Besredka's which these workers confirmed was that the intravenous dose of living paratyphoid B bacilli required to kill rabbits prepared with bile was only about one-tenth of the lethal dose for unprepared animals. Webster[179] showed in 1922 that roughly the same percentage of mice acquired protection against an orally administered challenge with *S. typhi murium*, irrespective of whether the vaccination route adopted had been peroral (the natural route of infection in mouse typhoid), intrapleural, or intraperitoneal. In other words, the immunity developed was of a general rather than a local nature. Similar conclusions were later reached by Greenwood, Topley and Wilson.[85] In 1923, Webster[180] noted marked individual variation, both in respect to susceptibility to infection, and of agglutinin production in survivors, among mice infected *per os* with a fixed dose of *S. typhi murium*. His findings emphasized the need for employing large groups of animals in experiments of this type, particularly when (as in much of Besredka's work), the host species was normally refractory to the microörganism under test. Pursuing enquiries into the factors influencing host susceptibility in mouse typhoid, Webster[181] found that 0.25 cc. of ox-bile given by mouth to mice slightly increased the infection rate, but caused obvious discomfort and diarrhea. Lesser amounts had no effect upon susceptibility, while 0.5 cc. either killed the mice within 24 hours, or made them extremely ill, with severe diarrhea. Webster therefore conceded Besredka's claim that treatment of rabbits with bile promotes absorption of bacteria given *per os* by injuring the intestinal mucosa, but emphasized the excessive toxicity of the dose required to enhance intestinal permeability in mice. Calmette,[24] in 1923, and Enlows,[69] in 1925, pointed out the impossibility of administering to man the doses of ox-bile (2 to 3 cc. or more per kg.) used by Besredka as a preliminary to oral vaccination in rabbits.

Further inquiries were launched into the necessity of using bile for effective oral immunization of laboratory animals by Sédan and Herrmann,[162] who reported in 1924 that typhoid infection could be produced in fasting guinea pigs by intramuscular injections of virulent living culture. Similar infection could be induced by much smaller doses injected into nonfasting animals, after 10 cc. of bile had been given orally on each of the 2 days preceding the inoculations. Control animals which had fasted 70 hours were sacrificed, and showed a bile-stained intestinal mucosa, with desquamation, hemorrhage, and absence of mucus—effects resembling, on a smaller scale, those

produced by administration of bile. Burke and Barnes[23] in 1926 confirmed certain of Besredka's findings as regards immunization of rabbits by mouth with typhoid bacilli, but did not use bile. In their hands, oral vaccination proved less effective than subcutaneous inoculation in stimulating agglutinin development; and they considered that mere ease of administration, and reduced toxicity, did not warrant substituting the oral for the subcutaneous method. Again without resorting to bile, Kosmodemiansky[98] reported in 1928 that heat-killed cultures of *S. paratyphosa B* might prove fatal when given by mouth in large doses to pigeons. Surviving pigeons withstood challenges up to 6 M.L.D. given orally. Immunity sometimes developed after a single ingestion of vaccine, about the 16th day; but the serum showed neither agglutinins, bacteriolysins, nor protective power.

Besredka's Oral Vaccination Methods Applied to Man. In certain parts of the world with an unduly high typhoid fever incidence, some form of Besredka's "vaccin bilié," or "bili-vaccin," was by now being tried out on man. Since reports on field trials of oral immunization with typhoid-paratyphoid vaccine have seldom referred to antibody production, their consideration will be deferred. Findings on the time and degree of antibody development in experimental subjects will first be reviewed.

Hoffstadt and Thompson[91] recorded in considerable detail, in 1929, the development of agglutinins in about 100 university students. Two-thirds of them took 1 cc. of T.A.B. vaccine (about 1 billion typhoid bacilli) before breakfast, for 3 successive days, in a glass of water. The remainder did likewise, but swallowed 2 bile capsules before the first dose. There were no unfavorable reactions. Agglutinins against *Eb. typhosa* developed in 88.5% of the whole group, to titers of 1:600 in some instances. Titers were highest 4 to 5 weeks after vaccination, and then declined irregularly for several months. Slightly more agglutinin production was shown by the bile-prepared subjects, and a small control group given the customary series of subcutaneous inoculations did still better. The same authors[92] also found complement fixation and precipitin reactions in an equally high percentage of a smaller group of similarly treated students. Hoffstadt and Martin[93] reported results of the same order following replacement of the fluid vaccine by gelatin capsules containing concentrated T.A.B. vaccine, mixed with ox-bile in a starch matrix. Two of 36 subjects reported abdominal reactions. The agglutinin titers attained a lower average level than in the groups taking fluid vaccine.

Good agglutinin responses were also reported, in 1930, by Pijper and Dau,[127] who gave 40 billion heat-killed, dried *Eb. typhosa*, preceded by a bile pill, on 3 successive days to 9 female patients (7 of them native Bantus) in the Pretoria Mental Hospital. Agglutinins of O type developed in all the treated subjects, the highest titers being reached in about 3 weeks. Pijper[126] had previously referred to the high frequency of O-agglutinin formation in South Africa. The paper by himself and Dau suggests the more likely explanation that their techniques promoted unduly high readings; for they used live antigen suspensions, and recorded reactions to the last trace, in most of their tests. Krause-Shimkin,[99] in 1931, tested 46 persons for typhoid agglutinins, before and again three to 5 weeks after receiving 3 daily doses of T.A.B. vaccine, with bile, by mouth. Forty persons who had some time previously received typhoid vaccine, either parenterally or by mouth, developed O-agglutinin titers up to 1:1000, the increases ranging to 20-fold in the former, and to 5-fold in the latter. Six persons not previously vaccinated or infected, and without initial agglutinins, developed O titers to 1:100 or 1:200. These reports of O-agglutinin response in orally vaccinated subjects are interesting, in view of the greater immunologic significance attached to O as compared with H agglutinins. Topley and Wilson[174]

have suggested the importance of knowing whether Vi agglutinins can be detected in orally vaccinated subjects.

Finally, Ruge[154] reported in 1932 giving 22 men on 3 successive days *per os* a tablet containing typhoid bacterial lysates. Four weeks later, 8 showed agglutinin titers of 1:400, 8 had no agglutinins, and the remainder showed intermediate titers. Two subcutaneous injections of vaccine were then given, and the average titer for the group rose to between 1:1600 and 1:3200: whereas controls receiving only the 2 subcutaneous inoculations developed an average titer of 1:400 to 1:800. He concluded that oral vaccination might produce a specific preparedness of the tissues, manifested in a more rapid and abundant production of antibodies on subsequent exposure to the same antigen.

Many workers were much less successful. For instance, in 1924 Achard and Bloch[1] detected no agglutinins, but a delayed and irregular development of complement-fixing bodies, in 8 persons who had received orally, along with a cholagogue, 2 cc. of standard T.A.B. vaccine before breakfast on 6 successive days. Similar negative results occurred in 5 women, who swallowed 2 bili-vaccine pills daily for 3 days—double the dose then generally used.

Poor serologic responses were likewise recorded by Rosa,[140] who fed 33 persons 4 T.A.B. tablets on 2 successive days before breakfast. A month later, 1 person showed typhoid agglutinins to a titer of 1:200, and all the others had none. Pfeiffer and Lubinski[125] also fed dried typhoid bacilli to 12 students, and 2 to 6 weeks later found no circulating agglutinins or bacteriolysins. In 1937, Lewin, Gear and Landau[106] published a comparative study of the antibody formation, involving 60 healthy South African natives. One group received subcutaneously 2 doses of typhoid vaccine, with 1 week between doses; another group was similarly treated with Grasset's typhoid "endotoxoid"; and the third group received oral typhoid vaccine in pill form, on 3 successive mornings, a bile pill being given the previous evening. The products were all prepared from the same strain of *Eb. typhosa*. Samples of blood were collected 2 and 4 weeks respectively after the last dose of vaccine, and the sera tested for H and O agglutinins and complement-fixing bodies. A feeble antibody response was shown by the orally vaccinated group. The average O agglutinin titer merely doubled, rising from roughly 1:20 to 1:40, by contrast with an average rise to around 1:400 in persons receiving the antigens subcutaneously.

More recently, Naumer and Nerb[114] gave a commercial preparation of typhoid vaccine orally to 6 individuals, 3 of them children under 12 years of age. A capsule containing 10 billion heat-killed bacilli was taken fasting, without bile, on 3 successive mornings. There were no reactions but only 1 subject showed a slight rise in agglutinin titer. Elledge, Kennedy and Cumming[68] also reported findings on a large group of adults, 1200 of whom received T.A.B. vaccine subcutaneously, while 850 took it by mouth in capsules, again without bile, on 3 mornings before breakfast. Reactions were commoner among the subcutaneously inoculated, but nausea and "upset stomach" were noted by some who swallowed capsules. Slide agglutination tests carried out on 50 persons in each group showed some agglutinin response among all injected with vaccine, whereas 35 of the orally vaccinated group did not respond, and the remainder had very low titers.

To sum up this section, ingestion of large doses of killed typhoid-paratyphoid bacilli may lead to antibody formation in laboratory animals and man. When antibodies do appear, they are at lower titers than those customarily noted after parenteral inoculations of similar antigens. Apart from conventional antibodies, and any general immunity which these may signify, animal experiments suggest that some degree of local immunity may be induced in the intestinal wall, by ingestion of either living or heat-killed

vaccine; but the adequacy of any protection afforded by these immunity mechanisms appears dubious. Bile may facilitate absorption of some orally administered antigens; but in the small doses employed clinicaliy its effects must differ from those which follow treatment of experimental animals with the large amounts used by Besredka.

Prophylactic Administration of T.A.B. Vaccine Orally to Man. Most of the clinical reports on oral vaccination of man against enteric infection are unsatisfactory since immunization was usually begun in the decline phase of epidemics. Further, where oral and parenteral routes were compared, factors conditioning the choice of route in given individuals could have greatly affected their exposure risks. Only brief outlines will therefore be given of certain outbreaks hitherto adduced as evidence of the efficacy of oral immunization.

Dubarry[61] reported the effects of vaccination in a serious outbreak of typhoid fever among 1000 prisoners of war at Toulouse in 1914. About 6 weeks after the epidemic began, 282 persons received 3 injections of vaccine, while 373 took without ill-effects 2 of Lumière and Chevrotier enterovaccine pills. During the 1st fortnight after vaccination began, 3 persons receiving the injected vaccine developed typhoid fever. No further cases occurred thereafter. The author naively states that the most energetic prophylactic measures were instituted simultaneously with vaccination.

A number of similar reports appeared in France and South Africa soon after Besredka's work had attracted attention. Vaillant[177] first recorded a large-scale application of the bili-vaccination method to man. In 1921, a group of 6 villages in the war-devastated region of Pas-de-Calais were stricken with a typhoid fever outbreak. The living conditions were very squalid, with bad overcrowding, and contaminated wells. Vaccination was regarded as the sole weapon available against the epidemic; but in view of the general disinclination of the inhabitants to risk reactions, 1236 received the vaccine orally, and 173 subcutaneously, while 600 to 650 persons remained non-vaccinated. Among the non-vaccinated there were altogether 50 cases of typhoid fever (21 after vaccination had begun in the area); among the subcutaneously vaccinated, 4 cases developed within 5 to 12 days after the last injection; while only 3 cases developed among the orally vaccinated, all within 10 days after ingestion of the vaccine. Although Vaillant found these figures encouraging, he erred in calculating the incidence rate for the non-vaccinated on the basis of total cases since the epidemic began, instead of cases subsequent to commencement of vaccination. When this correction is made, the non-vaccinated case incidence was 3.2%, a figure approaching the 2.3% incidence for the subcutaneously inoculated group. The superficially impressive difference between these rates, and the 0.17% incidence calculated for the orally vaccinated, is statistically insignificant in the face of lack of epidemiologic evidence pointing to equality of exposure in the groups compared. Calmette[24] early pointed out that proper assessment of the efficacy of typhoid vaccine taken by mouth would entail carefully controlled experiments over a period of years, involving large numbers of subjects, in areas of typhoid fever endemicity.

The Pas-de-Calais episode was prototype to others to which similar criticisms apply. Besredka[16] himself reported a typhoid epidemic at the military college of La Flèche in 1923. After the peak had passed, 253 students received T.A.B. vaccine subcutaneously, and 268 physically weaker ones took his bili-vaccine by mouth. Among the former group, 10 contracted the disease within 20 days after inoculation; of those vaccinated by mouth, 5 fell ill within 11 days. Besredka regarded this outbreak as comparable to a laboratory experiment, since all the students lived under the same conditions, and concluded that the bili-vaccine was at least as efficacious as T.A.B. vaccine given by injections. However, only 2 sub-

cutaneous inoculations appear to have been given, on successive days, which are not optimal conditions for this method. Moreover, according to Calmette,[24] this outbreak was water-borne, and since the suspected water supply was doubtless banned, such cases as occurred thereafter were presumably due to chance encounters with *Eb. typhosa*. The numbers involved at La Flèche were again not large enough to furnish comparable indices of immunity with so inconstant an exposure risk.

Gauthier[79] reported, in 1924, his experiences in Greece during the expulsions from Asia Minor, when the value of subcutaneous T.A.B.C. vaccine was incontestably demonstrated on over 650,000 refugees. A limited trial of oral immunization was made on some 1200 nonrefugees living in Athens and Salonica. In the ensuing 6 months, no typhoid cases occurred among this group. Also, the inhabitants of villages where typhoid outbreaks had occurred ingested 3 billion polyvalent, heat-killed organisms, without bile, on 3 successive mornings before breakfast. Some 3500 persons in all took the vaccine, and only 5 among them developed typhoid fever.

A large-scale trial of bili-vaccine pills was reported from an oil center town in Rumania by Cantacuzène and Panaitescu[39] in 1925. After 54 typhoid cases had occurred, an intensive vaccination program was launched. A total of 8673 received T.A.B. vaccine subcutaneously; 2286 took bili-vaccine by mouth; 5575 controls remained untreated. Of 45 subsequent cases, 3 arose in the inoculated group (0.035%), and 6 in the orally treated (0.26%). Unfortunately, these figures did not include cases among the many persons, vaccinated or not, who left the city during the outbreak.

The poor level of sanitation and of community intelligence among the native populations of South Africa mines and villages led to extensive trials of oral vaccination there. From Pretoria[132] it was reported in 1927: "Natives most strongly object to having needles stuck in them. . . . They raise no objections to taking any amount of medicine by mouth. . . . In none of the thousands of natives orally vaccinated has any malaise occurred." In 1923, Pirie and Orenstein[128] administered orally to about 3500 native employees of a Witwatersrand gold mine, where 35 cases of typhoid fever had broken out, a teaspoonful suspension of 40 billion polyvalent typhoid bacilli, with an ox-bile pill, on 3 successive days. Of 20 subsequent cases, 16 occurred among 3500 vaccinated employees (0.57%), and 4 among 100 nonvaccinated (4%). Boyd[20] reported in 1928 that bili-vaccine pills had been given to 2400 Europeans and colored persons in Pretoria, without ill-effects, and with no instance of typhoid fever in persons to whom the pills had been given "within a few days of exposure to infection." Boyd also stated that 14 intestinal carriers of typhoid bacilli had given negative stool cultures following oral vaccination. Cluver[42] in 1929 wrote even more enthusiastically of the reduced incidence of typhoid fever in various gold mines after oral vaccination. Many of these reports from South Africa are of little significance, since all mines were then under pressure, to which they responded in varying degrees, to improve their sanitation standards. Incidence rates for different years were compared when oral vaccination cannot have been the only variable; and there was occasional evidence of invalid statistical calculations.

The foregoing optimistic claims from the field must be contrasted with the 1931 report of De Mello *et al.*,[51] who found no differences between the typhoid fever incidence shown by orally vaccinated and unvaccinated persons in Portuguese India. Moreover, other reports from South Africa summarized by Lewin,[105] in 1938, were unfavorable to oral immunization. His own statistics showed that oral vaccination failed to reduce the typhoid incidence over a period of several years. Indeed, the annual attack rate of verified typhoid fever in gold mine laborers was actually higher among the orally immunized—a

fact ascribed to the greater infection risks faced by the more usually vaccinated underground laborer, as compared with the surface worker. Grasset,[84] in 1939, stated moreover that the typhoid morbidity and mortality rates in the Rand gold mines during 1934–1936 were 7 times higher among nearly 80,000 immunized employees than among roughly 150,000 who had received his endotoxoid subcutaneously.

Bacillary Dysentery. Shiga's isolation of the causal microörganism of the most severe form of dysentery, in 1898, stimulated interest in the feasibility of vaccination against this disease. Various methods were suggested for reducing the extreme toxicity of even heat-killed cultures, including admixture of the bacilli with immune or normal serum, and vaccination *per os*. Preliminary results in animals were encouraging, but interest faded until reawakened by the work of Besredka, which led to extensive trial of oral immunization against Shiga dysentery in man. The types of experiments conducted closely resembled those described in the foregoing section. The results obtained, and the fallacies inherent in the conclusions often drawn therefrom, were likewise analogous. The relevant literature will therefore be reviewed in less detail.

Results of Animal Experimentation. At the International Congress in Moscow in 1904, Gabritschewski[76] briefly alluded to animal experimental findings which suggested the possibility of oral immunization of man against acute intestinal infections, such as dysentery, typhoid and cholera. On the same occasion, Zeitlin[189] reported having himself swallowed 6 doses of heat-killed Shiga bacilli, ranging up to 10 mg. of bacterial bodies, over a 26 day period. There were no ill-effects, and no antibodies developed. Hida and Toyoda[90] recorded agglutinin and bacteriolysin formation in guinea pigs fed with peptic and tryptic digests of dysentery bacilli, but found no such response to ingestion of heat-killed cultures. Shiga[164] immunized rabbits by feeding heat-killed cultures of his bacillus. When challenged intravenously with toxin, his animals showed protection against intestinal effects which killed controls in 1 to 2 days, but they died later with spinal paralysis. Shiga also alluded to good results with the vaccine in mental hospital patients.

Chvostek,[41] in 1908, recorded irregular immunity development, unrelated to antibody formation, in rabbits fed with large doses of killed or living Shiga bacilli. Parenteral inoculations proved more effective. Dopter[58] also showed that mice could be similarly immunized by mouth against 1 to 2 M.L.D. of Shiga bacilli. Excessive doses of vaccine killed the mice, and small doses failed to immunize. Immunity developed only after 10 to 12 days, and did not last beyond 30 days. Later, Dopter[59] stressed the impracticability of the oral route in man, in view of the large doses needed (15 gm. daily), as judged by the requirements in mice.

In 1910, Dopter and Repaci[60] noted that the inflammatory and ulcerative changes found post mortem in the large intestine of rabbits given live cultures of Shiga bacilli by mouth, or subcutaneously, were similar to those present in the natural disease of man. Several years later, Besredka[12] reopened study of this question by confirming Dopter and Repaci's findings. He also reported that agglutinins appeared after a single large ingestion of such bacilli; and that rabbits thus treated were vaccinated against living bacilli injected intravenously. In view of the innocuity of the procedure, he urged its trial prophylactically in man. Soon afterwards, Besredka[14] showed that his observations on rabbits applied also to mice. He further noted that rabbits which had developed serum agglutinins after receiving heat-killed Shiga bacilli *per os* soon lost these agglutinins, and could not be stimulated to produce more by additional ingestions of bacilli. Nevertheless, such animals exhibited a solid immunity, which he concluded was entirely localized in the intestinal mucosa, against a severe chal-

lenge dose. According to Besredka, in seeking to provoke such immunity one should avoid production of antibodies, since these are only acquired at the price of severe reactions. Bile facilitated development of immunity, but was not deemed essential here, because the desquamating action prerequisite to absorption of the bacilli was achieved by the organisms themselves.

Kanai,[95] in 1921, endeavored to reproduce Besredka's results in rabbits, but obtained only slight immunity after oral vaccination. Ingestion of large doses of *Sh. dysenteriæ*, living or dead, had no effect upon the health of rabbits. Cultures of enfeebled virulence may have been used. Dumas and Combiesco[63] also failed to produce intestinal lesions by feeding live Shiga bacilli to fasting rabbits. However, paralyses and cecal lesions resulted from giving toxic 8 day culture filtrates by mouth to rabbits and guinea pigs. Half the animals died. The survivors were resistant to 4 M.L.D. of living bacilli injected intravenously. In 1925, Enlows[69] reported a 57% survival rate in a group of 106 rabbits vaccinated by mouth, and then challenged intravenously, as against 70% in a similar group subcutaneously immunized. Bile was shown to be unnecessary, and possibly disadvantageous, in promoting oral immunization. The oral vaccinating doses contained 100 to 200 times as many heat-killed organisms as were given subcutaneously, but caused fewer fatalities (30% as compared with 43%). Not only do these reports in general dispute many of Besredka's claims regarding the efficacy of immunization by mouth, but Enlows' results indicate the potential dangers of perorally administered Shiga bacilli.

Recently, Cooper and Keller[44] provoked high degrees of immunity in mice against *Sh. sonnei* by gavage and voluntary drinking. About 300 billion living or killed *Sh. sonnei* given in 4 days induced resistance to 16 to 160,000 M.L.D. of homologous organisms suspended in mucin and injected intraperitoneally. Smaller dosage gave rise to lesser degrees of immunity. Powell and Jamieson[131] obtained similar results in mice from oral administration of various types of dysentery "endotoxoids," prepared by Grasset's method of adding formalin to repeatedly frozen and thawed bacilli. Endotoxoid derived from 20 billion bacilli given orally twice daily for 5 days proved harmless to mice, and induced immunity against injected challenges of 10 to 100 M.L.D. of culture—a degree of immunity comparable to that noted by others in mice immunized parenterally.

Reports on Administration of Shiga Dysentery Vaccine per Os to Man. In 1922, Nicolle and Conseil[117,118] presented reports on the successful vaccination of man against Shiga dysentery by Besredka's method. They selected dysentery and brucellosis as suitable infections for experiments in man, on the grounds of their controllability. Since native Tunisians were usually resistant to Shiga dysentery (a fact ascribed to the drinking of polluted waters since infancy), they used subjects of European extraction. Over a 5 day period, 2 volunteers ingested four 100 billion doses of heat-killed Shiga bacilli. Just over 2 weeks later, 1 of the subjects swallowed 10 billion, and the other 20 billion living Shiga bacilli. Both remained perfectly well. Two unvaccinated controls given the same dosages contracted typical dysentery, verified by stool culture.

Gauthier[78] reported in 1924 the results of bacillary dysentery prophylaxis through oral vaccination, carried out in Greece under the auspices of the League of Nations. In all, some 30,000 persons received 3 daily doses of the vaccine, each comprising 3 billion heat-killed Shiga and Flexner bacilli. No cases of dysentery were subsequently reported among the vaccinated. Several examples were cited of epidemics which ceased abruptly and completely after oral vaccination. In a refugee camp of 4000 persons, contaminated well water had given rise to 400 cases of proven Flexner dysentery, with 50 deaths. After two-thirds had received

oral vaccine, dysentery occurred only among the unvaccinated. Another episode involved transferring 340 orally vaccinated refugees from a healthy camp with a good water supply, to another camp with a contaminated supply, where an epidemic was already in progress. None of the migrants developed dysentery.

Less impressive data were offered, also in 1924, by Anglade,[3] reporting on a severe dysentery outbreak affecting the garrison and neighboring population in Versailles; and by Antonovsky[4] describing outbreaks in several Petrograd institutions. Both reports committed the same error of comparing the incidence of dysentery among the orally vaccinated, when the epidemic was past its peak, with the total incidence of cases among the unvaccinated. Moreover, very little bacteriologic work was done. Again in 1924, Pascal[122] sought to testify in favor of oral immunization by comparing the incidence of Flexner dysentery in an institution whose inmates had not been vaccinated, with the postvaccinal incidence of Shiga dysentery in the following year.

CHOLERA. Inquiries into the pathology of *V. choleræ* infections in laboratory animals, initiated by Metchnikoff, led to the conception of enterotropism, vigorously sponsored later by Sanarelli. According to Sanarelli, living cholera vibrios administered intraperitoneally to guinea pigs,[156] by mouth or subcutaneously to young[157] and adult[159] rabbits, by the same routes to man,[160] and by mouth to newborn puppies,[158] inevitably made their way to the mucosa of the small intestine. Masaki,[111] and more recently Solarino,[167] came to similar conclusions. Besredka, less convincingly, ascribed enterotropic properties also to typhoid, paratyphoid and Shiga dysentery bacilli. There are but few reports on oral immunization against cholera; perhaps because the immunity induced by cholera vaccine subcutaneously is too incomplete and transitory, and reactions too slight, to justify risking still more uncertain degrees of protection against so serious a disease.

Fairbrother[70] has summarized the various cholera antigens which had been employed parenterally prior to Besredka's work. Until then, the only noteworthy attempt at oral immunization with cholera vaccine was made by Hida and Toyoda,[90] who fed guinea pigs filtered peptic and tryptic digests of *V. choleræ*. Serum agglutinins and bacteriolysins appeared after 8 to 14 days. The applicability of Besredka's theories and methods to experimental cholera infections in animals was reported by Masaki[111] in 1922, Glotoff,[81] in 1923, Horowitz-Wlassowa and Pirojnikowa,[94] in 1926, and by Pfeiffer and Lubinski,[125] in 1930. Masaki found rabbits and guinea pigs completely refractory to living vibrios taken by mouth. After bile sensitization, rabbits became susceptible to cholera endotoxin, and succumbed in 1 to 2 weeks from repeated ingestions of large doses of living vibrios. Animals surviving a minor cholera infection were able to resist a normally fatal intravenous dose of living vibrios. Masaki concluded that immunity to cholera infection in rabbits and guinea pigs is localized in the intestine. Glotoff, working mostly with rabbits, and Horowitz-Wlassowa and Pirojnikowa, using guinea pigs, recorded essentially similar results and conclusions. Pfeiffer and Lubinski fed rabbits heat-killed cultures of *V. choleræ*. Serum bacteriolysins appeared, but agglutinins only developed in bile-treated animals.

Calmette[24] expressed doubt whether oral vaccination against cholera in man would be feasible, but alluded to trials of the method then being conducted in Russia. Such brief reports as have been available from that country and elsewhere seem too unconvincing and contradictory to require mention.

TUBERCULOSIS. Calmette and Guérin,[28] in 1906, contended the pathogenesis of tuberculous infection in man and animals involved passage of the bacilli through buccal and intestinal mucous membranes to the mesenteric ganglia. From here,

bacilli might be carried by the lymphatic circulation into the right heart, and thence to the lungs. According to them, even pulmonary tuberculosis was intestinal in origin rather than droplet-borne. This view, which was propagated by these workers for many years, led them to investigate the immunizing efficacy of avirulent tubercle bacilli when given by mouth to young animals. Preliminary experiments on calves and kids, with a heat-attenuated human strain, were sufficiently encouraging to evoke the prediction that so inoffensive a mode of vaccination might be applied to infants.

In 1920, Calmette and Guérin[29] reported that an initially virulent bovine strain of *M. tuberculosis*, first isolated by them 12 years before had become so avirulent that at least 44,000 bacilli could be safely given intravenously to man. By 1924, the strain had become completely avirulent for all animal species, and a vaccine prepared from it had rendered young calves resistant to large doses of virulent bacilli.[30] Calmette, Boquet and Nègre[26] soon afterwards showed that young rabbits and guinea pigs could be successfully vaccinated against experimental tuberculosis by repeated oral administration of B.C.G.; and in 1925 Wilbert[182] reported that chimpanzees and other anthropoids could be similarly protected against infection from apes with which they were cohabiting.

In 1928, Calmette *et al.*[31] published figures showing the apparent prophylactic value of B.C.G. given orally during early infancy. The merits and limitations of the immunity conferred on humans by B.C.G. vaccine are still controversial, and it is obviously difficult to assess the relative efficiency of a given route of administration as long as the actuality of immunization by any route remains in dispute. Among the multitude of papers published on B.C.G. in recent years, it is impossible to be confident of selecting the most authoritative. However, 2 impressions are outstanding: (1) that the evidence favoring B.C.G. is making converts at a quickening pace; and (2) that the oral route is mostly being abandoned. There are exceptions to this trend. De Assis,[50] reviewing 17 years' experience with B.C.G. vaccine in Brazil, reported good results, with certain provisos, from the oral route. Again, Gomez Ullate[83] has commended from Spain the simplicity, economy and effectiveness of the oral route, and advocates routine vaccination at birth, with revaccination by the same route at 1 and 3 years of age, and subsequently on changes of environment. On the other hand, Cantonnet Blanch *et al.*[40] repudiated the oral route as giving unsatisfactory results in Uruguay. They ascribed this to frequent rejection of the vaccine through vomiting. A recent British review[112] summarized the problem thus: "Statistical data derived from oral vaccination of the newborn are unsatisfactory because of uncertainty of B.C.G. infection, lack of control, and difficulty of separating specific from non-specific factors. But there can be little doubt that the vaccinated groups—perhaps 2 to 3 million in all—have shown a decidedly lower mortality from tuberculosis during childhood years than non-vaccinated infants in the same communities."

THE BACTERIAL EXOTOXINS ABSORBED THROUGH THE ENTERON. Two important bacterial exotoxins share the property of exerting specific effects upon the central nervous system after absorption from the enteron. These are the toxins of *Cl. botulinum*, and the enterotoxin produced by some strains of staphylococcus. A few references will also be made in this section to the immunizing efficacy of scarlet fever toxin, and of diphtheria toxin, when taken by mouth.

As long ago as 1891, Ehrlich[67] reported that mice could be immunized *per os* against as many as 200 to 800 M.L.D. of the plant toxin *ricin*. Their blood serum showed specific antitoxic properties. Ehrlich's findings, confirmed in 1937 by Allin,[2] suggested that some bacterial toxins might resist destruction by the gastro-intestinal enzymes, and exercise some antigenic effect after absorption.

Tetanus and diphtheria toxins, and snake venom, were found to be largely destroyed in the upper part of the alimentary canal. The discovery by van Ermengen, in 1897, of the causal bacillus of botulism, and Barber's demonstration, in 1914, that staphylococcal growth products might cause gastro-intestinal disturbances when swallowed by man, furnished examples of bacterial toxins apparently unaffected by the digestive juices.

Botulinum Toxin. Tchitchkine[170] attempted, in 1905, to immunize rabbits by mouth against botulinum toxin, but produced only a slight degree of active immunity, while their serum acquired no protective power. Tani[168] reported, in 1934, that several doses of a formalinized toxin given daily to guinea pigs produced circulating antitoxin. However, animals receiving identical dosages subcutaneously developed a much higher antitoxic titer. Recent work in this laboratory by Wood[185] showed negligible immunity development in mice given Type A botulinum toxoid intensively by mouth.

Staphylococcus Enterotoxin. Barber[9] noted that farm residents who had frequently consumed milk from a cow excreting staphylococci from the udder were resistant to gastro-intestinal disturbances suffered by visitors who drank the same cow's milk. In 1931, Dack, Jordan and Woolpert[49] found that subminimal reacting doses of filtrate from a food poisoning strain of staphylococcus, given orally to volunteers almost daily for several weeks, provoked little or no immunity. Dolman[55] reported, in 1943, that subjects reacting violently to enterotoxin on multiple occasions over several months, showed no evidence of acquired immunity. These findings were later extended.[56] Volunteers who had retained an unaltered susceptibility to ingested enterotoxin became refractory for several months to at least 5 minimal reacting doses after a series of subcutaneous injections of enterotoxin.

Scarlet Fever Toxin. Dick and Dick[52] noted the inefficiency of oral immunization with scarlet fever toxin. To 19 Dick-positive children, they gave *per os* 12 to 16 daily doses, increasing from 4 to 16 cc. of undiluted toxin. For comparison, some 200 susceptibles received the customary series of 5 graduated doses subcutaneously. Among those treated orally, 73% were Dick-negative after an average total of nearly $8\frac{1}{2}$ million S.T.D.; while of those inoculated subcutaneously with a total of 135,500 S.T.D. each, 93% showed a negative reaction. Later,[53] they employed coated tablets containing concentrated toxin in amounts up to 2 million S.T.D. per tablet. These were fed to 131 susceptibles, ranging in age from 1 to 36 years, who were skin-tested before and after the series had been taken. In a group of 102 persons, 95% were Dick-negative 2 weeks after the last dose. It was recommended that a total of 75 million S.T.D. should be taken within 3 weeks. Scarlatinal rashes, vomiting and diarrhea often occurred when the tablets disintegrated in the stomach. Carefully prepared tablets only occasionally caused cramps and transient rashes.

Diphtheria Toxin. Chvostek[41] fed diphtheria toxin to 10 guinea pigs, but only 1 survived subcutaneous challenge with 2 M.L.D. of toxin. On the other hand, Breton and Petit[21] fed guinea pigs cultures of *C. diphtheriæ*, attenuated or killed by heat, and the animals later withstood inoculation of more than 2 M.L.D. of toxin. The inconsistencies apparent between these 2 reports areresolvable by assuming that small amounts ofdiphtheria toxin, adsorbed on bacterial bodies, might survive contact with intestinal enzymes, and cause a slight production of antitoxin. Dumas, Combiesco and Baltiano[64] killed guinea pigs and rabbits by oral admin istration of very large doses ofdiphtheria toxin.

Bousfield[19] reported, in 1945, that increased circulating antitoxin developed in humans who had sucked pastilles containing diphtheria toxoid. The method was ineffective as a primary immunization stimulus. He thought at least 3 pastilles, of 100 Lf content, should be sucked daily

for 1 week if a marked rise in antitoxin titer were sought. Four subjects who swallowed gelatin capsules containing the equivalent amounts of toxoid showed no increased antitoxin. The antigenic stimuli were therefore held to take place between the lips and the lower end of the esophagus, rather than through the gastro-intestinal mucosa.

THE MISCELLANEOUS MICROORGANISMS. *Pasteurella Pestis.* Fornario[75] claimed that heat-attenuated plague bacilli, given in 2 doses *per os* or *per rectum* at 10 to 14 days interval, incited immunity to subcutaneous challenge with virulent cultures in two-thirds of a small group of rabbits and guinea pigs. Serum antibodies, notably opsonins, also appeared. Noc[119] and also Leger and Baury[103] recorded inconclusive experiments of a similar nature.

Pseudomonas Æruginosa. Courmont and Rochaix[47] administered heat-killed, 8 day broth cultures of *Ps. æruginosa* (*Bacillus pyocyaneus*) to rabbits by rectal instillation. Three doses of 100 cc., given with a long cannula, at 5 day intervals, were well tolerated. One month after the last dose, the animals were challenged intravenously with virulent homologous culture. They developed fever, diuresis and loose stools, but recovered after a few hours. Controls died.

The Brucella Group. Nicolle and Conseil[117,118] gave 2 volunteers *per os* within 5 days a series of 4 doses of 100 billion killed *Br. melitensis.* A fortnight after the last dose they had no detectable agglutinins. Challenged subcutaneously with live culture, they remained well, whereas a similarly inoculated control subject became ill 17 days later, with verified brucellosis.

Dolman and Hudson[57] reported that 4.5% of over 5000 blood specimens from inhabitants of an area where Bang's disease was prevalent completely agglutinated brucella suspensions in a titer of 1:20 or higher. Since most of these specimens came from persons with no history of brucellosis, the agglutinins presumably developed as a result of frequently repeated ingestions of dead or living *Br. abortus.* An unpublished experiment involving 2 volunteers showed that a single, large dose of killed *Br. abortus,* taken in milk, resulted in no increased titer of serum agglutinins.

Exclusively Non-human Animal Pathogens. Gaiger[77] reported, in 1909, that young buffaloes which had repeatedly drunk large doses of *hemorrhagic septicemia* bacilli survived a normally fatal subcutaneous challenge with virulent culture. The immunity was incomplete and transitory, but was held to explain the natural resistance to infection shown by old animals in endemic areas. Baldrey[8] carried these inquiries further, and confirmed Gaiger's claims. Since 1 out of 14 bulls died of infection from the oral vaccination, the procedure was not regarded as safe.

Baldrey also attempted unsuccessfully to vaccinate 12 bulls against *rinderpest* by administering with their food or water 2 small doses of blood from infected animals. Calmette[25] later quoted Van Saceghem as claiming successful protection following a single ingestion of fecal suspension, prepared from an artificially infected animal treated with antiserum. These references to rinderpest, and a curious observation by Repetto[134] that rats and mice fed normal nervous tissue became resistant to subcutaneous injection of *rabies* street virus, exemplify the paucity of the literature on oral immunization against virus infections.

Duff[62] showed that trout developed immunity to *fish furunculosis* after repeated feedings (64 or more) with hatchery food containing the causal pathogen, *Bacterium salmonicida.* Specific agglutinins developed in many of the vaccinated fish, and when exposed to infection their mortality was 25% against 75% for untreated fish.

THE PYOGENIC COCCI. These microorganisms have certain common features, apart from morphology, to justify their being grouped together in this final section. The alimentary canal seldom serves as their portal of entry into the host; the defence mechanisms against them appear

mainly phagocytic; and they frequently become secondary invaders, especially in respiratory tract infections.

Staphylococcus. Calmette and Petit[32] showed, in 1907, that virulent staphylococci administered to rabbits by stomach tube, soon appeared in the blood stream and killed the animal within 24 hours. Given rectally, the sequence of events was slower. Osteomyelitis appeared either spontaneously, or after trauma. Courmont and Rochaix[48] could incite only partial immunity in rabbits by rectal instillations of *Staphylococcus pyogenes aureus.* Combiesco and Calalb[43] also tried unsuccessfully to immunize rabbits orally, with or without use of bile, against intravenous challenges with staphylococci. Winzeler[183] reported better results in guinea pigs given many large doses of staphylococci by stomach catheter. A majority of animals fed living organisms later survived challenge with 3 M.L.D. of culture, but the killed organisms provoked inferior degrees of immunity. Coincident use of bile did not improve the immunity acquired.

Streptococcus. Tchitchkine[171] reported rabbits frequently died of streptococcal septicemia when fed live cultures, but took heat-killed cultures with impunity. Rabbits surviving such ingestions showed no acquired immunity against challenge 6 months later with living organisms. Winzeler[183] fed guinea pigs large doses of *Strep. hæmolyticus,* living and dead, without significant immunity development. Kolmer and Amano[97] also failed to immunize rabbits against intracisternally injected virulent streptococci, despite 30 daily ingestions of 5 to 10 cc. of culture, living or heat-killed. Moreover, no agglutinins developed.

Pneumococcus. The oral immunization against pneumococci has yielded more impressive results. In 1927, Kimura[96] fed many large doses of heat-killed Type I pneumococci to mice, and found 5 out of 8 withstood 100 M.L.D. of injected live culture. Kolmer and Amano,[97] in 1932, reported irregularly immunity development in rabbits following 30 daily administrations of heavy doses of pneumococci by stomach tube. Some animals survived intratympanal injection of homologous culture in amounts fatal to controls. The best protective results were obtained from ingestion of living Type I pneumococci. The authors ascribed the comparative rarity of pneumococcal meningitis in human paranasal sinus infections to immunity derived from continually swallowed organisms.

Meanwhile, in 1926, Ross[144] published the first of several important papers on oral immunization against pneumococci. He used white rats in his experiments, since mice responded irregularly, and rabbits negatively. Rats fed repeatedly on tissues from rats which had died of Type I pneumococcus infection survived intraperitoneal challenge with 1000 to 10,000 M.L.D. of homologous organisms. It was suggested that these findings might account for the infrequency of lobar pneumonia from pneumococcus types commonly present in normal throats. Almost equally good results were reported by Ross[142] from repeated feedings with living Type I pneumococci. Equivalent dosages of heat-killed organisms, or of culture filtrates, were ineffective, but hydrochloric acid-killed organisms[144] gave good protection. Ross[150] also showed immunity thus acquired was of variable, but on the whole brief duration, many large ingestions of preferably living organisms being needed to procure its persistence for 1 to 2 months; occasionally up to 4 months.[143] But lapsed immunity could be restored by a single feeding. Pneumococci desiccated after mechanical disruption, or dissolved in bile salts,[145] and also Type I specific soluble substance,[147] produced almost equally good protection when fed to rats.

Ross[146,148] soon extended his observations to cover Types II and III pneumococcus. He also showed[149] that most of the soluble specific substance ingested by rats was excreted unaltered in the feces. He concluded tentatively that certain cells of the gastro-intestinal tract were

responsible for their increased resistance. However, in 1934, he[151] reported the presence of protective antibodies (against pneumococci given intraperitoneally to mice) in the blood of rats orally immunized against Type I pneumococcus, and suggested positive protective antibody tests in human sera might indicate increased resistance. Similar protective antibodies were detected[151] in rats fed with Types II, III and IV. Finally, he[153] detailed the results of feeding 63 individuals with mixed Types I, II and III preparations. Within 2 to 3 days after 6 heavy feedings, about 75% of the group formed protective antibodies against Type I, and about 60% against Types II and III. Agglutinins were not detected in these or preceding experiments. Since the antibody level reached (1 cc. of serum protecting a mouse against 5000 M.L.D. of homologus pneumococci) approximated that reported in sera from pneumonia convalescents, its attainment seemed to indicate a generalized specific resistance.

Heterophil and Common Cold Antigens. In 1925, Powell[129] reported that heterophil (Forssman) antigen administered by mouth to rabbits, in such forms as hen, sheep or goat red cells, evoked high titers of heterophil antibody (anti-sheep hemolysin). Bailey and Shorb,[7] in 1931, demonstrated circulating heterophil antibody in rabbits fed intensively with heat-killed Type I pneumococci, but higher titers followed a single intravenous injection. These animals were also immune to inoculations of living pneumococci. Rockwell, Van Kirk and Powell[137] asserted, in 1935, that heterophil antibodies were "effective" against all pneumococcal types, as well as certain streptococcal strains, and several other common secondary invaders in colds. Having noted raised titers of heterophil antibodies in the blood of convalescents from acute colds, they deduced that a "non-type specific heterophil active immunity" against colds might be provoked by oral vaccination. A capsule of 100 billion heat-killed pneumococci and streptococci was taken on 7 successive days, and thereafter once weekly throughout the season, by 500 patients. Fewer colds than usual were experienced by this group; but as was pointed out,[65] the control group was largely invalidated by inclusion of persons with a lower average susceptibility. These authors further stated contradictorily that "bile destroys the heterophil antigen," and that "the vaccine was high in bile-resisting heterophil antigen." Later,[138] other common respiratory tract organisms were added to the vaccine, with even more favorable results.

Rockwell and Van Kirk[135,136] sought to correlate heterophil antibody in orally vaccinated subjects with acquired immunity to colds; but their protocols did not indicate prevaccinal titers, although a brief reference in the earlier paper suggests as wide a range of antibody in unvaccinated persons, as was subsequently found in those who took the vaccine. Powell[130] even developed a serologic method for assaying the adsorbing power for heterophil antibody of mixed cold vaccines. He also applied to such vaccines the method described by Ross[151] for determining the protective power of the serum of rats fed with Type I pneumococcal preparations. Thus, although provocation of non-type-specific immunity in humans had been previously urged, a test for cold vaccine was now recommended which measured only type-specific immunity developed by rats.

Working along somewhat different lines, Thomson *et al.*[173] administered orally to a few volunteers phenol-killed cultures of *H. influenzæ* grown symbiotically with *Anæromyces bronchitica*. Quite marked reactions ensued, and some serum agglutinins and mouse-protecting substances developed. They deduced that a combined vaccine, including pneumococci, streptococci, and *N. catarrhalis*, also grown symbiotically, would have even greater prophylactic value against complications of colds and influenza. The authors kept up their own agglutinins by swallowing a weekly dose of mixed vaccine.

Numerous proprietary preparations of semisecret composition continue to be advocated here and there for oral immunization against ailments ranging from intestinal disorders to rheumatism and coryza. The best that can be said of these is that they pay tribute to the faded prestige of Besredka's doctrines or demand faith in the dubious significance of heterophil antigens.

Discussion and Conclusions. "I had satisfied myself long ago . . . that, though typhoid vaccine may be absorbed by way of the intestinal canal, it is only badly and incompletely absorbed, and above all that its action is uncertain. . . . I do not think it is necessary to go any further than that, for science never sanctions a more complicated and more uncertain method being employed where a simple and certain one, such as subcutaneous inoculation, is available. And I would submit that the idea that a *via media* can be found between the antique system of prescriptions, with doses taken t.i.d. before or after meals, and scientific applications of bacteriology, and the idea that that *via media* can be found in the administration of vaccines by mouth, ought steadily to be put away from us." Thus spoke Wright,[187] pioneer of vaccine therapy, in 1910. Over 30 years later, his long-time associate, Fleming,[73] found little cause to modify this opinion, and dismissed the issue in less peremptory but briefer terms: "In certain communities this method has been very popular owing to the ease of administration. Extensive observations have shown that in the enteric group of diseases some immunity can be established by the oral administration of vaccine, but it is not the best method."

Although a review of the literature has shown how aptly and fairly these 2 quotations summarize the status of oral immunization today, the matter cannot be left just there. For even if the inferiority of this route has always been recognized by the prescient and the experienced, its potentialities apparently remain enticing enough to secure a perennial, sporadic interest. There seems no need to recapitulate the flimsiness of the experimental and epidemiologic supports upon which the protagonists of oral immunization lean. But certain question remain to be answered. First, what is the likelihood of methods being devised for evoking more predictable and higher immunity responses to orally administered antigens? Secondly, is there a type of local, intestinal immunity, independent of conventional antibodies, which in some circumstances it is desirable, and even preferable, to provoke? Thirdly, are there certain overriding advantages of the oral route—simplicity, cheapness and innocuity, for instance—which, despite inefficiencies, occasionally warrant its adoption? These question will be dealt with in order.

1. Feasibility of Inducing More Satisfactory Responses to Oral Antigen. Once swallowed, antigens become subject to successive hazards to their integrity, and handicaps to their absorption. Before any intimate contact with the intestinal mucosa is achieved, the antigen faces exposure to hydrochloric acid and pepsin; to bile, trypsin, and other proteolytic enzymes of intestinal or even bacterial origin—all liable to be encountered in concentrations and at intervals governed by such variables as the subject's diet, bowel motility, and emotional and physical health, as well as perhaps the state of the weather. Covering the tract is a layer of mucus which presents simultaneously a mechanical barrier, and a menstruum in which leukocytes can better pursue and engulf entangled microörganisms. Goldsworthy and Florey,[82] and Florey,[74] have discussed other antibacterial properties of this intestinal mucus.

Antigens surviving these preliminary obstacles impinge upon a mucous membrane of high selectivity, whose underlying mechanism eludes analysis. Considerations of size, motility, or lability cannot wholly account for the different capacities to enter the blood stream shown

by *Eb. typhosa* and other members of the salmonella species; nor for the remarkable discrimination exemplified in human susceptibility to ingested botulinus toxin of Types A, B and E only; and in the innocuity of staphylococcus alpha-toxin[54] and beta-toxin[55] taken by mouth, in contrast to the effects of the enterotoxin. The total effect of all these characteristics is to ensure that persons of normal health and habits seldom or never absorb more than trivial amounts of unaltered antigen. This concept readily accounts for the frequent lack of circulating antibody after ingestion of antigen.

However, the impermeability of the intestinal mucosa to proteins is by no means complete. For instance, Makaroff[110] induced specific anaphylactic states in guinea pigs by repeated heavy feedings with protein; Hektoen, Kanai and Dragstedt[89] used precipitin tests to demonstrate absorption of thyroglobulin in dogs fed on beef thyroid; and the Seegals[163] provoked sterile inflammation in the actively sensitized rabbit eye by introducing homologous antigen into the stomach. Arnold[5] found that when typhoid, coliform and other bacilli were injected with egg-white into the duodenum of dogs, they passed freely into the thoracic duct, and reached the liver and mesenteric lymph nodes in large numbers. These observations on dogs were extended[115,166] to cover absorption of various microörganisms introduced by mouth, intraduodenally or rectally. Fischer[71a,72] also reported that yeast ministered orally and rectally was absorbed into the portal circulation.

In 1927, Walzer[178] showed by means of the Prausnitz-Küstner reaction that some fish and egg protein taken by man in physiologic amounts was customarily absorbed undigested. Ratner and Gruehl[133] reported similar observations, and suggested that protein absorption in man might usefully serve the purpose of keeping the body immunized against habitual protein diets. Arnold, viewing the foregoing findings from another standpoint, contended[5] that alterations in permeability of the enteron occurred naturally in response to various environmental stimuli, and might be correlated with certain epidemiologic phenomena. Later[6] he stated: "The intestinal mucosa is . . . a permeable body covering and the reticulo-endothelial network within the splanchnic region is the real barrier against systemic invasion." Alluding to experiments by Peterson, Müller and Boikan,[124] in which marked splanchnic reactions were the first tangible effects of continuous intravenous perfusion of dogs with *E. coli*, Arnold urged that specific immunity might be simply and physiologically acquired by applying splanchnic stimuli, after orally administered antigen had passed the enteron and was fixed in the splanchnic reticulo-endothelial system. The evocation of these stimuli would certainly provide a fertile field for necromantics.

Proofs of protein absorption explain the many reports of irregular antibody production following ingestion of antigen. They account also for such observations as those of Tenbroeck[172] on specific antitoxin in the blood of intestinal carriers of *Cl. tetani*. Furthermore, they strike at the very roots of Besredka's doctrines, that the host's sole defence against bacterial invasion from the enteron was an intact gastro-intestinal mucosa, which could be rendered impregnable by assimilation of "antivirus," a soluble component released from ingested bacteria by phagocytosis. But oral immunization now faces multiplied theoretical difficulties; for the destiny of that variable portion of ingested antigen which may traverse the mucosa, is largely indeterminate. Most particulate elements, mainly transported by leukocytes and macrophages, will reach the mesenteric lymph glands; and the remainder, along with soluble components, will go to the liver directly. The work of Ehrich, Harris and co-workers[66,86*] on the rôle of the lymphocyte in immunity, indi-

* For the fate of particulate antigens in relation to antibody formation, see Harris, T. N., and Ehrich, W. E.: J. Exp. Med., **84**, 157, 1946.

cates that any ingested antigens arriving intact in the splanchnic lymph glands might thence call forth efficient antibody responses. The relatively good results from oral B.C.G. vaccination perhaps derive from the high proportion of unaltered antigen reaching this area. As for the liver, despite its importance to immunity in bacteremic conditions,[38] its detoxifying functions must also be reckoned with; under the circumstances in question, any large contribution of antibody from that organ could hardly be anticipated. The fate of an antigen, and the kinetics of antibody formation, have recently been studied by a promising new technique, involving the injection into mice of purified tobacco mosaic virus, tagged with radiophosphorus.[107,155] Application of such methods to ingested antigens should prove illuminating. Meanwhile the assumption seems warranted that oral dosages large enough to ensure regular passage of effective amounts through the intestinal mucosa into the splanchnic depôts, apart from their inconvenience, would entail a heavy risk of overflowing the splanchnic barrier into the systemic circulation, with possible reactions of great severity. Calmette and Breton[27] forewarned against such dangers long ago, with respect to tubercle bacilli.

2. Significance of Local Intestinal Immunity. The largely unconfirmed nature of Besredka's laboratory results, and the doubtful efficacy of his bili-vaccines in man, illustrated earlier in this review, seem inevitable in the light of the foregoing considerations. Indeed, the more extreme and dogmatic aspect of his local immunity doctrines were soon rejected by the orthodox. But there has remained a sufficient afterglow of enthusiasm to inspire the occasional neophyte, or to tempt the uncritical manufacturer; their discouragement seems in the public interest. Hence certain other fallacies should be briefly exposed here.

First, a few comments on the use of bile, whose toxic effects in Besredkan dosages have already been described. Not only are breaches in the intestinal mucosa unnecessary for passage of antigen, they need not even facilitate this process. Gaiger[77] failed to induce hemorrhagic septicemia in young buffaloes fed contaminated grass so coarse that stomach abrasions were bound to result; Boone *et al.* (*Proc. Soc. Exp. Biol. and Med.*, **29**, 113, 1931) noted no increased absorption of bacteria after injuring the duodenal mucosa. On the other hand, several workers have reported increased permeability of the gastro-intestinal walls after subjecting them to pH changes such as might result from the cholagogue effects of bile. Again, *E. coli* was noted[116] in the mesenteric lymph nodes and liver of dogs soon after intragastric administration of saponin, which has a similar effect to bile on surface tension. In the small doses advocated for promoting oral immunization in man, any action of bile is obscure, but may relate to the acceleration of stomach evacuation described by Pannett and Wilson[121] in 1921.

Secondly, the enterotropic concept, which loomed large in Besredka's writings, is a relative, teleologic term. Harvey[88] referred to a septicemic type of typhoid fever, without intestinal symptoms, in which the tonsils and throat served as portal of entry. Even complete intestinal immunity could furnish no protection here. Benians[10] reported that in experimental coli-typhoid septicemias, the organisms migrated into subcutaneous fixation abscesses induced by gum tragacanth, this suggesting a possible "mucotropism" rather than any selective organ affinity. Besides, no matter how large the dose of vaccine ingested, it is hard to conceive that all the innumerable crypts and tortuosities of the gastro-intestinal mucosa could thereby be rendered impervious to subsequent infection. On the other hand, it is clearly irrelevant to attempt vaccination of the entire enteron, with a view to securing impenetrability, against organisms whose elective affinities appear to be directed, for instance, towards the respiratory tract, and whose portal of

entry usually lies therein. Only some measure of general immunity could be expected to cope with such contingencies as the foregoing; and the oral route provides only the vaguest assurance of its attainment.

The question remains whether local, intestinal immunity should be sought, either as manifesting an essentially different form of protection, or as providing a supplementary depôt for storage and mobilization of antibodies. Besredka's disparagement of antibodies stemmed mainly from observing that orally vaccinated animals sometimes showed firm protection despite absence of circulating agglutinins, and from instances of immunity having failed in man despite the presence of antibodies. His views served the useful purpose of activating controversy and inquiry respecting the rôle of antibodies, but the evidence is now overwhelming that he erred in denying their significance. According to current immunologic concepts, antibody globulin is believed to be synthesized within such cells as macrophages and lymphocytes adjacent to the site of deposition of antigen.[33,36] Antibody and serum globulin production are subject to similar limiting influences,[37] and their molecules exhibit the same life span.[161] The gist of these views may be cogently applied to the present argument, in the following words of Cannon:[35] "It is not the fact of antibody concentration in the blood stream at any particular moment that is so significant in resistance as that there has been a retention of the capacity of the antibody producing tissue to become reactivated and thereby enabled to resist bacterial agents against which they have once been immunized." This reactivation process, or anamnestic response, may be extremely prompt, and has been discussed at length by Cannon.[34] Mention should also be made here of Wright's impressive recantation,[188] in 1942, of former views on the dependence of the phagocytic activity of leukocytes upon serum opsonins; and of his conviction that phagocytosis may be rapidly stimulated, even *in vitro*, by vaccination. In sum, although Besredka's local immunity concept found early support[80] outside discipular circles, modern views on immunity mechanisms point to the probability that the phenomena he observed were at least partly due to antibody globulin held mainly intracellularly, and partly to enhanced phagocytosis. This hypothesis is supported by such observations as those of Torikata and Imaizumi[175] that guinea-pigs fed on *E. coli* or staphylococcus had for a short period higher opsonin titers in fluid extracts of their intestinal mucosa than in their blood serum, while the reverse was true in animals subcutaneously immunized. Favilli and McLean,[71] on the other hand, went so far as to ascribe the major features of local immunity, at least as noted in the skin, to a reduction in the tissue permeability associated with early inflammatory changes, rather than to any specific immune response.

3. *No Present Justification for Even Occasional Resort to Oral Immunization.* The only hypothetical advantages accruing from oral immunization then appear to derive from a temporary and uncertain concentration of antibody, unreleased from cells in the intestinal wall, and in their residual anamnestic potentialities. But such protective mechanisms could only be exercised against agents seeking entry by the alimentary canal. Moreover, the protection would be unevenly distributed, so that through some portal in the long defence line the invader might pass unchallenged, and emerge into relatively defenceless territory. Following parenteral vaccination, a much more uniform and widespread distribution of antibody is brought about by the blood stream.

As an alternative to sole reliance upon oral immunization, the findings of Ruge[158] suggested its supplementation by subcutaneous inoculations. But no benefits would be thus conferred that are not forthcoming, with far greater certitude, from growing acceptance of the need for for an initial series of injections, with "booster" or "recall" stimuli similarly

administered. Fast-improving public attitudes towards hypodermic inoculations should encourage endeavors to reduce the toxicity, and fortify the antigenicity, of products, and to determine how the immunizing capacity of the skin[176] can be used to best advantage. These efforts should not be undermined by any pandering to dim apprehensions, or confused by dissemination of quasi-scientific literature whose esoteric jargon the average practising physician cannot follow, let alone the layman; and which often enough relates to products incapable, however administered, of conferring specific protection. Unless and until some entirely unforseen immunologic principles be established, licensing authorities should accept the responsibility of discountenancing distribution of products intended for oral immunization.

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PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF JANUARY 20, 1948

The Blood Pressure of Rats Subjected to Auditory Stimulation. ELEANOR H. YEAKEL, PH.D., and HENRY A. SHENKIN, M.D., with assistance from S. M. MCCANN and A. B. ROTHBALLER (Dept. of Research Surgery, Univ. of Penna. and Wistar Institute). After a years exposure to the sound of a blast of compressed air, 5 minutes daily for 5 days a week, the average systolic blood pressure (determined with a tail plethysmograph) of 25 gray Norway rats rose from an initial value of 113 to 154 mm. in the last 2 months. In 17 control animals, an increase from 124 to 127 mm. for the group was found over the same period. Sixteen of the air-blasted rats (64%) and 2 of the controls (12%) had mean blood pressures of 150 mm. or more at the close of the experiment.

Monthly averages indicated that the rise in blood pressure of the experimental rats tended to occur in the 9th month after air blasting was begun. The average of all readings for each rat before and after the rise in its blood pressure showed a mean change of +29 mm. for the group.

Direct measurements of the blood pressure with a Hamilton optical manometer,* made on 11 of the control and 13 of the experimental rats, did not agree with plethysmograph readings on the intact unanesthetized animals. In general, the systolic pressure of the air-blasted rats was lower. When the 2 methods were used on the same animal under ether, the systolic readings agreed closely. In experimental rats, the average diastolic pressure was 89 mm., as compared with 78 mm. for the controls; and values of 90 mm. or more were recorded for 8 of the former and 1 of the latter. The differences are significant statistically.

The results suggest that the auditory stimulus increased peripheral resistance and raised systolic and diastolic pressure.

Quantitative Measurement of Regional Circulation by the Clearance of Radioactive Sodium. SEYMOUR S. KETY, M.D. (Dept. of Pharmacology, Univ. of Penna. and Metabolic Service, Phila. General Hosp.) A freely diffusible substance injected into a tissue will be carried away from the site of injection at a rate largely determined by the local circulation. If the substance be the radioactive isotope of sodium (Na^{24}), the quantity remaining in the tissue and hence the clearance rate can readily be measured continuously by means of a shielded Geiger-Mueller counter at a fixed distance from the injection site. It was mathematically predicted and experimentally found that the locally injected sodium disappears along an exponential curve: $Q = Q_0 e^{-kt}$, where Q = quantity of sodium remaining at time, t, Q_0 = initial quantity of sodium injected, k = the tissue clearance constant. This constant is readily obtained as the slope of the straight line which results when Q (in counts per minute) is plotted semilogarithmically against time. It is further demonstrable that k bears the following relationship to blood flow: $k = \frac{F}{S}\theta$, F = tissue blood flow as cc. per gm. per min., S = sodium space of the tissue as cc. per gm. θ, numerically somewhat less than unity = an overall diffusion factor depending on diffusion distances, capil-

* We are indebted to William A. Jeffers, M.D., of the University Hospital, for performing the determinations.

lary interface, fluid filtration and absorption. Thus the clearance constant, although not a rigid measure of total blood flow, is an accurate and quantitative measure of the effectiveness of the local circulation including not only blood flow but other mechanisms responsible for local homeostasis. In tissues where diffusion occurs rapidly and is not a limiting factor, the clearance constant approximates the blood flow. This technique was employed to study the circulation in the human gastrocnemius muscle. There was satisfactory agreement among values obtained in different subjects. A tourniquet about the thigh invariably brought the clearance constant to zero or practically zero. Reactive hyperemia, exercise and locally injected mecholyl produced a marked increase in the clearance constant, while local administration of epinephrine slowed the clearance markedly. Values for flow calculated from the clearance constants are in general agreement with other estimates for muscle blood flow in man. The convenience of this technique and its ability to yield quantitative values for effective circulation may make it a useful clinical method.

Thermodynamic Analysis of the Relative Effectiveness of Narcotics. FRANK BRINK, PH.D. (Johnson Foundation, Univ. of Penna.). Narcosis is a cellular process apparently reaching equilibrium. For a system of molecules in equilibrium the partial molal-free energy, or chemical potential, of a given type of molecule is the same in all phases of the system. Therefore, in accordance with the definition of thermodynamic activity (Lewis and Randall), this quantity is also constant for each molecule in each phase at equilibrium. These thermodynamic quantities for a narcotic in any part of a narcotized cell are therefore known if they can be determined in the external solution.

The relative effectiveness of numerous narcotics on many different preparations were compared by calculating the activities corresponding to the measured concentrations in the external solution or gas. If the narcotic concentrations in the aqueous phase were given, the activities were calculated by means of the measured activity coefficients published by Butler (*Trans. Faraday Soc.*, September, 1936). If the data were in the form of partial pressure in a gas phase in equilibrium with the narcotized preparation then the activity was calculated as the ratio of this quantity to the vapor pressure of the pure narcotic at the same temperature. By these procedures the pure narcotic is employed as the standard state of each substance.

The results show that for many narcotics of different chemical structure acting upon a variety of cell types, equal degrees of effect are produced at equal thermodynamic activities. This conclusion contrasts sharply with findings of Ferguson *Proc. Roy. Soc.*, B, **127**, 387, 1939) and of Badger (*Nature*, **158**, 585, 1946) both of whom emphasize the progressive increase, in an homologous series of compounds, of the thermodynamic activity at which narcosis occurs.

The available data indicate that for the same narcotics either of these orderly relations can occur, depending upon the type of cell studied and the kind of cell function that is being measured.

The rule that for many narcotic substances equal degrees of narcosis are produced at equal thermodynamic activities, though severely restricted by such exceptions, is common enough to be regarded as the simplest law of narcosis. It is suggested that this rule is an important guide for the development of an adequate theory of the molecular mechanism of narcosis.

Action of Narcotics on Synapses Compared to Axons in Sympathetic Ganglia. J. M. POSTERNAK, M.D.,* and M. G. LARRABEE, PH.D. (Johnson Foundation, Univ. of Penna.). The action of narcotics on

* Fellow of the Swiss Foundation for medico-biologic studies.

synaptic functions were compared with effects on axonal conduction by recording impulses transmitted through perfused stellate ganglia of cats. The postsynaptic action potential in the inferior cardiac nerve following preganglionic nerve stimulation measured the number of cells responding to synaptic excitation; the action potential of the cervical sympathetic trunk measured the number of Type B fibers conducting impulses through the stellate ganglion without synapse. The effectiveness of a narcotic was measured by the concentrations required to depress the action potentials in these nerves to 50% of their control amplitude.

Chloretone and sodium pentobarbital exhibited preferential action on the synaptic pathway, which was depressed by concentrations respectively about one-seventh and one-eleventh those which equally depressed conduction over B fibers. Chloroform and ethyl ether also depressed preferentially the synaptic pathway at concentrations about one-third those depressing conduction over B fibers to the same extent. Both these narcotics caused detectable depression at concentrations equalling those in blood for human surgical anesthesia.

By contrast, ethyl urethane and ethyl alcohol had no preferential action on synapses. Actually axonal conduction was depressed by concentrations lower those equally depressing the postsynaptic response.

The preferential action on the synaptic pathway developed progressively in the homologous series of aliphatic alcohols, the ratio of required concentrations being a linear logarithmic function of the number of carbon atoms. For methyl alcohol, a 50% reduction of the action potential of the B fibers was reached with about one-half the concentration required for the same reduction of the postsynaptic response; for octyl alcohol, a 5 times higher concentration was required for B fibers than for the synaptic pathway.

For all these narcotics, a correlation is shown between their effectiveness and their differential action: the lower the required narcotic concentration, the higher is the preferential action for the synaptic pathway.

For a given effect on the synaptic pathway, thermodynamic activities were nearly equal for ethyl ether, chloroform and the several alcohols, while molar concentrations varied nearly 19,000-fold. For effect on B fibers, the thermodynamic activities for the alcohols increased 10-fold with number of C atoms, from C_1 to C_5.

Analysis of Skin, Muscle and Brachial Arterial Blood Temperatures in the Resting Normal Human Forearm. H. H. PENNES, M.D. (Psychiatric Inst. of New York). Forearm skin and muscle, brachial arterial blood and rectal temperatures were measured by radiometric and thermocouple techniques in 64 subjects, 30 to 60 years in age (patients of the Neurological Institute, New York, without evidence of neuromuscular disease of the arms). Subjects were basal, nude except for a small sheet over the hips and lay on an ordinary hospital bed with the right forearm supported in air. Air and wall temperatures were identical and ranged from 25 to 27.5° C.;* air relative humidities from 45 to 75%; linear air velocity was always below 20 feet per second.

(*a*) Mean brachial arterial blood temperature was 36.68° C., mean maximum forearm temperature 36.52° C. in 10 subjects. In each of these subjects the brachial arterial blood temperature was higher than maximum forearm temperature by amounts ranging from 0 to 0.36°.

(*b*) The longitudinal skin temperature gradients along the superior surface of the long axis of the forearm averaged 0.03° C. per cm. in 14 subjects, a negligible value in the mathematical analysis.

(*c*) Thermal distributions in 17 subjects around the circumference of the forearm at the experimental plane (8 cm. distal to olecranon process of ulna) were spati-

* All temperatures are in ° C.

ally non-uniform; average maximum difference between any 2 forearm points was 1.2° C. for the group. In 14 subjects, occlusion of blood flow both through and below the experimental plans showed that these non-isothermal distributions were influenced by the venous return from the distal part of the forearm.

(*d*) Deep tissue temperature distributions in 9 subjects through the entire transverse axis of the forearm at the experimental plane were approximately parabolic; lack of perfect circular symmetry of the individual curves precluded logarithmic analysis. The mean experimental deep tissue temperature curve was therefore approximated by curves giving the theoretical distributions of temperature in solid tissue cylinders with uniform rates of heat production and volume flow of blood. The mathematical analysis combined the general differential equation of heat flow in homogeneous, isotropic conductors, the Fick principle of physiology and the Newton Cooling Law. Closest approximation of the experimental data was obtained with substituted values of local tissue heat production of 0.0001 gm. cal. per cm.^{3} per second and blood flow of 0.0002 to 0.0005 gm. per cm.^{3} per second. If the volume flow of blood and the other necessary data are obtained; then the rate of local resting forearm tissue heat production may be obtained by this analytic method.

BOOK REVIEWS AND NOTICES

APHASIA: A GUIDE TO RETRAINING. By CAPT. LOUIS GRANICH, U. S. Army Med. Admin. Corps. Appendix in collaboration with SGT. GEORGE W. PRANGLE, U. S. Army Med. Corps. Pp. 108. New York: Grune & Stratton, 1947. Price, $2.75.

IT is recognized that aphasic manifestations do not exist alone, since brain injuries often cause "personality changes, some degree of general mental deterioration, and physical handicaps, as well as specific mental disabilities outside of the language functions." Indeed, these may be more obvious than is the aphasia. The following important topics, with their subdivisions, are considered: The Typical Speech Disabilities, Receptive Aphasia, Alexia and Related Disorders Affecting Reading, Agraphia and Related Disorders Affecting Writing.

The retraining of 8 selected cases is given, though no definite outline is followed, and only the significant features are discussed. Case 1 showed an injury to the left parietofrontal region; part of the technique employed was through copying drills, left hand writing which was rapidly developed, training in articulate sounds, passive moulding of mouth parts, and mirror imitation of mouth movements. In about a month the subject was able to pronounce any sound, he wrote fairly well but scrambled long words, and rhythm was used, beating out time for each syllable. After prolonged patient training, much progress was made, though at the last recording of the case, difficulty was still experienced in sentence formulation. This volume is a valuable contribution to the complex problem of retraining aphasics.

N. Y.

REHABILITATION THROUGH BETTER NUTRITION. By TOM D. SPIES, M.D., Univ. of Cincinnati Studies in Nutrition at the Hillman Hosp., Birmingham, Alabama. Pp. 94; 50 figs. Phila. and London: Saunders, 1947. Price, $4.00.

THIS volume is a recapitulation of the author's extensive experience with deficiency disease. In it he reiterates the criteria upon which diagnosis of deficiency of the separate vitamin fractions is made and describes the therapeutic regimen he has employed in relief of such deficiencies. Most of this information is now generally available to the practicing physician due to the pioneering work in the nutrition field to which Dr. Spies himself largely contributed. The present volume adds nothing new, either in information or point of view, but does offer a convenient summarization of Dr. Spies' experience.

K. E.

STANDARD METHODS OF THE DIVISION OF LABORATORIES AND RESEARCH OF THE NEW YORK STATE DEPARTMENT OF HEALTH. By AUGUSTUS B. WADSWORTH, M.D. Foreword by GILBERT DALLDORF, M.D. 3d ed. Pp. 990; 105 figs. Balt.: Williams & Wilkins, 1947. Price, $10.00.

THIS edition contains over 300 pages more than did the 2d edition. While the sections and chapters are very much the same, in most cases they have been expanded to include recent material. The section on mycology has been enlarged considerably and a new section "Examination of samples concerned with restaurant sanitation" has been added. No mention is made of the use of thioglycollate medium for the sterility testing of biological products containing organic mercurials for preservatives as is required by the U. S. Food and Drug Administration. The book still remains a valuable one for public health laboratories.

H. M.

A MANUAL OF FRACTURES AND DISLOCATIONS. By BARBARA BARTLETT STIMSON, A.B., M.D., Med. Sc.D., F.A.C.S., Ass't Prof. of Clinical Orthopedic Surgery, College of Physicians and Surgeons, Columbia Univ. New York.: 2d ed. Pp. 223; 98 ills. Phila.: Lea & Febiger, 1947. Price, $3.25.

THIS short text is in its 2d edition and represents in a remarkably small space a concise, clearly written handbook on the treatment of fractures and dislocations. Dr.

Stimson has had a wide experience with the effects of trauma on the human body, both on the Fracture Service at the Presbyterian Hospital and in the British Army in World War II. The book can be highly recommended to the medical student and general practitioner as the best short organized guidebook on this subject available.

P. C.

CHEMISTRY AND METHODS OF ENZYMES. By JAMES B. SUMNER, Prof. of Biochemistry, Cornell Univ., and G. FRED SOMERS, Plant Physiologist, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y. 2d ed. Pp. 415; 10 figs. New York: Academic Press, 1944. Price, $6.50.

PROGRESS in the fields of enzyme chemistry has made necessary a new edition of this valuable book. Approximately 50 pages of additional descriptive material have been added, and some whole sections have been rewritten. The major additions have been in the sections upon general properties of enzymes, phosphatases (esterases), carbohydrases, and the broad group of enzymes concerned with carbohydrate metabolism, namely, phosphorylases, transphosphorylases, phospho-isomerases and phosphomutases. All other sections have been brought up-to-date, and errors that appeared in the 1st edition have been corrected. The authors make no claim that this is a complete source book of enzyme information. In fact, it was not written for this purpose. Detailed directions are given for the preparation and measurement of the activity of many enzymes by procedures that have been checked thoroughly in their laboratories. The book continues to be the best available for everyone requiring a concise reference volume to the whole field of enzymes and their chemistry.

H. V.

THE CHEMICAL COMPOSITION OF FOODS. By R. A. MCCANCE, M.D., PH.D., F.R.C.P., and E. M. WIDDOWSON, B.Sc., PH.D., Dept. of Medicine, Univ. of Cambridge. 2d ed. Pp. 156. Brooklyn, N. Y.: Chemical Publishing Co., 1947. Price, $3.75.

THIS is the second American edition of a widely used English handbook. Besides tabulating the composition of the various common foods, as purchased, data are also given for them after cooking, with detailed receipes for preparing the various cooked foods that have been analyzed. The two major sets of tables list the percentage and per ounce composition of each listed food. The tables contain information upon the protein, fat, available carbohydrate, calories, Na, K, Ca, Mg, Fe, Cu, P, Cl, and acid base values. In the English system of analysis the available carbohydrate is determined directly, and all values are computed as monosaccharide. Likewise, the authors compute caloric values by using the factors 4.1, 9.3, and 3.75 for protein, fat and carbohydrate. While the system is not the one ordinarily used in this country, this does not detract from the value of the book; a complete index of all foods analyzed facilitates its use.

H. V.

AN APPROACH TO SOCIAL MEDICINE. By JOHN D. KERSHAW, M.D., (Lond), D.P.H. Medical Officer of Health, Accrington. Pp. 329. Balt.: Williams & Wilkins, 1946. Price, $4.50.

THE first half of this volume is concerned with the development of society and has little to do with medicine of today, either in the treatment of disease or in public health aspects. It does, however, serve as a setting for Part 2—"Health and Sickness in Society," and this latter half of the book should be of interest to every physician.

The author's thesis is that we should not look upon disease as a pathological entity but as something that interferes with the practice of living. Then our objective becomes the rehabilitation of the social function of the individual and not anatomical restoration. The earlier in disease this objective is applied, the more effective it becomes; the longer it continues, the better the restoration. "The ideal restoration scheme will be integrated at one end with the hospital and at the other with industry."

It is further maintained that health is not merely freedom from disease but a state of "optimum functional efficiency." In order that the physician achieve this for his clientele, his understanding of environmental influences must be developed by a larger participation in research upon and investigation of many social problems such as food, housing, work, leisure, sex, genetics and education. There are a great many references to the literature, mainly of England, and suggestions for further reading.

S. B.

ATLAS OF CARDIOVASCULAR DISEASES. By IRVING J. TREIGER, M.D. Ass't. Prof. of Medicine, Univ. of Illinois, Chicago; in Charge of Cardiographic Department, Presbyterian Hospital, Chicago, etc. Pp. 180; 244 ills., 11 in color. St. Louis: C. V. Mosby, 1947. Price, $10.00.

THE author states that a cross section of common types of heart disease with autopsy findings is presented, with a selection of simple uncomplicated cases. Emphasis is placed on correlation of history, physical findings, roentgenogram and electrocardiogram for purposes of diagnosis and treatment. The atlas is divided into 6 sections: (1) the normal heart, (2) rheumatic disease, (3) arteriosclerotic heart disease, (4) hypertension, (5) syphilitic heart disease, and (6) congenital anomalies. Because of the elementary nature of the material presented, the atlas is doubtless intended for those who know little concerning cardiovascular diseases but wish to familiarize themselves with the findings in what may be called typical cases. Like most atlases, its greatest usefulness will be as a supplement to other forms of study. Cardiovascular diseases in their earlier and clinically more important stages do not yield striking pictures for reproduction in an atlas, and pathological material is more difficult to collect. For example, in this atlas there are no illustrations of the vitally important early stages of cardiovascular syphilis, although aneurysm and aortic insufficiency are adequately presented. Perhaps the most serious criticism that can be made is that the electrocardiograms include only one precordial lead.

C. W.

THE DIAGNOSIS AND TREATMENT OF DIARRHEAL DISEASES. By WILLIAM Z. FRADKIN, A.B., M.D., Assistant Attending Gastroenterologist, Physician-in-charge of Colitis Clinic, Associate Bacteriologist, Jewish Hospital of Brooklyn. Foreword by BURRILL B. CROHN, M.D. Pp. 254; 113 ills. New York: Grune & Stratton, 1947. Price, $6.00.

A BOOK such as this, stressing the importance of diarrhea and presenting the various conditions causing it, has long been needed. The first 70 pages include the anatomy and physiology of the digestive tract and general methods of treatment, diagnosis and prevention. Subsequently the diarrheal diseases are discussed individually as to their etiology, pathology, diagnosis and treatment. As stated in the preface, no attempt has been made to describe completely those conditions of which diarrhea is only a part. Thus, under the heading of "Diarrhea caused by hyperthyroidism," the other manifestations of that disease are not presented. This limitation, plus an unusual conciseness, has produced a relatively short, but all-inclusive, book. Fortunately, accuracy and completeness have not been sacrificed for brevity. Confusion or omission is evident in only a few instances: as in the therapy of amebic diarrhea where the use of two or more drugs is advocated but not explained, and in the therapy of several of the conditions due to toxic substances such as arsenic, mercury and muschroom poisoning. A bibliography is included at the end of every chapter for those desiring additional or more general information. The value of the book is enhanced by many excellent illustrations. A few more of these depicting, in greater detail, the microscopic appearance of the intestinal parasites would, however, not be amiss. In view of the prevalence and importance of diarrhea, this book should be of definite value to all internists and general practitioners as well as to students.

R. B.

CURARE, ITS HISTORY, NATURE AND CLINICAL USE. By A. R. MC INTYRE, Ph.D. M.D Prof. of Physiology and Pharmacology, College of Medicine, Univ. of Nebraska. Pp. 240; 25 ills. Chicago: Univ. of Chicago Press, 1947. Price, $5.00.

CURARE, once a laboratory curiosity, is now used frequently in clinical medicine. In this monograph McIntyre has gathered together practically every reference to the drug which has appeared from the 15th century through part of 1946. Such a complete summary of the literature should be of interest to the medical historian, neurologist, anesthesiologist, physiologist and pharmacologist. The early history of curare is presented in detail, as is a consideration of the botanical problems involved in identification of the various sources of the drug. One chapter is devoted to chemistry. There are also chapters on the action of curare on nerve and muscle, circulation and respiration, viscera and the central nervous system. The clinical use of curare is also

discussed. The author subscribes more or less to the cholinergic concept of neuromuscular transmission and interprets the action of curare in this light. This may not be acceptable to some readers. The possible rôle of histamine liberation in the picture of curare action and toxicity is omitted. A central nervous system action of curare is accepted on the basis of relatively inconclusive evidence. The bibliography is perhaps too complete. For example there are 178 references listed for 15 pages of Chapter 8. Many of these seem to be included for no particular reason. The format of the book is attractive. The paper is of good quality and the print easy to read.

R. D.

MILK AND FOOD SANITATION. By H. S. ADAMS, B.Sc. Division of Public Health, Minneapolis. Pp. 311; 65 ills. New York: The Commonwealth Fund, 1947. Price, $3.25.

WHEN the need for advice upon sanitation is recognized by a lay person the family doctor should be a reliable consultant. Whether he is or not, depends on many factors. An important one among these may well be the lack of a modern, attractive and factual text: one in which the clearly cut answers are so arranged and indexed that they can be easily and quickly identified. For practical information upon the best current practice in the sanitary production, handling and distribution of milk and other foods, this volume will satisfy every inquirer: the family doctor, the professional sanitarian, the person who wants to know. To have recognized the great general need for this book is an achievement in itself; to have satisfied a high ambition in its production is to aid all our communities toward better living. The first half of the book concerns milk, its history, its nutritive value, the public health problems connected with it and its economic importance. Then the author seems to take the health officer by the hand and lead him gently, but firmly and expertly, through all the details of building a high quality milk program and maintaining it. All the essentials of sanitary milk production, pasteurization and distribution are described in careful detail, with generous recognition and citation of the work of others. Nothing better has been written on milk sanitation since the late Milton J. Rosenau compared the bacterial content of the Washington City sewage with its market milk, and no flattery to the milk. The second half of the volume concerns food and its importance in the field of environmental sanitation. All details essential to the health officer in his control work are brought to the reader as vividly as though by television. Throughout the need for dependence upon educational methods is emphasized. In the appendix, information is given upon many matters such as: supplementary source maetrial, field equipment for inspectors, courses of instruction, the application of DDT, list of films relating to milk and the precision testing of high-temperature short-time pasteurizers.

A. H.

MEDICAL CLINICS OF NORTH AMERICA. Mayo Clinic Number. Blood Transfusions and Rh Factor. Pp. 785–1058. Phila.: W.B.Saunders, 1947. Price, $16.00 a year.

THE title of this issue is misleading, as only 5 articles deal with the subject. The remaining 15 articles cover a wide variety of subjects of interest to the general practitioner. The paper on radiophosphorus is well written and covers the limitations of this substance quite adequately. Of special interest is the article on duodenal ulcer by Thomas B. Magath, which again emphasizes the important conditions to be considered in the differential diagnosis of perforated duodenal ulcer. An article on atomic energy in medical practice generalizes on the medical values of atomic research and on some of the beneficial results that may be obtainable from substances with such destructive potentialties.

J. F.

FORENSIC MEDICINE. By KEITH SIMPSON, M.D., Lecturer in Forensic Medicine, Guy's Hospital, Examiner in Forensic Medicine, Univ. of London. Pp. 335; 114 ills. Balt., and London: Williams & Wilkins, 1947. Price, $4.50.

THIS book, though pocket size, is a comprehensive presentation of Forensic Medicine. It covers the entire field with emphasis on the pathological side, *i. e.*, autopsy procedure, wounds, signs of death, cause of death, blood stains, identity. The section on Toxicology is also well done, and includes

such new drugs as D.D.T. and the poison gases. Criminal Abortion and Infanticide are given full and adequate consideration.

In the field of Civil Malpraxis, Negligence, and Psychiatry, while hese subjects are well presented from the British standpoint, the difference between English and American practice not only becomes evident but is a serious drawback to the use of the book in this country. The classification of the insanities has been simplified and differs from that commonly used here. Such an important subject for forensic practice as the Psychopathic Personalities is not even considered. The age of consent, epilepsy as a defense for murder, the juvenile aspects of crime, and many other subjects, too, are presented purely from the English standpoint, as is Workmen's Compensation. While it may appear captious to point out these differences, if this textbook obtains the circulation among American doctors and students that it deserves, an edition incorporating American law and practice, would be well worthwhile. The book is fully illustrated, in color and in black-and-white, and has an excellent index. It "is designed to provide a brief and essentially practical guide to current teaching of Forensic Medicine." As such, it is the best in its field.

D. McC.

ATLAS OF HISTOPATHOLOGY OF THE SKIN. By G. H. PERCIVAL, M.D., A. MURRAY DRENNAN, M.D., and T. C. DODDS. Pp. 495; 376 color photomicrographs. Balt.: Williams & Wilkins, 1947. Price, $16.00.

THIS is a collaborative work by a dermatologist, a pathologist and the supervisor of the Laboratory of Pathology at the University of Edinburgh. Being an atlas, there is only a brief sketch of explanatory text preceding each section and so interpretations of pathologic processes have been sacrificed.

As to the illustrations, the first impressions are pleasing because they remind one of the familiar scenes under the microscope. More critical study, though, leads to the realization that many of them are inadequate either for the novice or the trained pathologist. It is true that the general patterning of the lesions is usually satisfactory; both in this respect and in sharpness of detail, the low power magnifications are fairly satisfactory, but high power ones are frequently worthless because finer cytologic changes are hazy. In short, some of the illustrations are not diagnostic even when the lesion is of the kind that is diagnostic, and most of them do not adequately portray the order of pathologic process, even, in the case.

Typographic errors are few: in some places the names of diseases are capitalized; Senear's name reads "Sennear." The more serious omissions of diseases consist in the blastomycoses, coccidioidal granuloma, senile keratosis, parafinnoma and bromide granuloma.

Errors in the actual content are few—one surmises that the seborrheic keratosis figured on page 311 is the hard nevus of Unna, and that the nevus pigmentosus on page 315 is a seborrheic keratosis. The illustrations on page 321 might equally be a blue nevus, such as is so inadequately portrayed on page 331, although the reviewer is aware of the different viewpoints as to pathologic concepts and terminology of the pigmented nevi.

The authors deserve credit for their enterprise and energy. They have succeeded in portraying the larger patterning aimed at, but when cytologic detail becomes involved there is much to be desired. In short, the book will be found useful only up to a certain point, both for the trained pathologist and the graduate student in dermatology.

F. W.

NEW BOOKS

American Pharmacy. Vol. II. Edited by RUFUS A. LYMAN, M.D., Director, School of Pharmacy, Univ. of Arizona. Pp. 379; ills. Phila.: J. B. Lippincott, 1947. Price, $7.00.

Hormones and Behavior. By FRANK A. BEACH, Prof. of Psychology, Yale Univ. Pp. 368. New York: Hoeber, 1948. Price, $6.50.

Surgical Treatment of the Abdomen. Edited by FREDERIC W. BANCROFT, M.D., F.A.C.S., Prof. of Clinical Surgery, New York Medical College, and PRESTON A. WADE, M.D., F.A.C.S. Pp. 1026; 457 ills., and 3 color plates. Phila.: J. B. Lippincott, 1947. Price, $18.00.

Teaching Psychotherapeutic Medicine. Edited by HELEN LELAND WITMER, PH.D. Pp. 464. New York: The Commonwealth Fund, 1947. Price, $3.75.

Medicine. Vol. I. The Patient and His Disease. By A. E. CLARK-KENNEDY, M.D., F.R.C.P., Physician to the London Hospital and Dean of the Medical School. Pp. 383. Balt.: Williams & Wilkins, 1947. Price, $6.00.

Pharmacology and Experimental Therapeutics. By HAMILTON H. ANDERSON, FUMIKO MURAYAMA and BENEDICT E. ABREU. Pp. 368. Berkeley and Los Angeles: University of California Press, 1947. Price, $6.50.

Kurze Klinik der Ohren-, Nasen- und Halskrankheiten. By DR. ERHARD LÜSCHER. Pp. 513; 201 ills. Basel: Benno Schwabe & Co., 1948. Gebunden Fr. 54.

American Medical Research Past and Present. By RICHARD H. SHRYOCK, PH.D., Prof. of History and Lecturer in Medical History, Univ. of Pennsylvania. Pp. 350. New York: The Commonwealth Fund, 1947. Price, $2.50.

Pathologisch - anatomische Untersuchungen über leberzirrhose bei Säuglingen und Kleinkindern (infantile Leberzirrhose) mit endemischer Häufung. By DR. MED. HERMANN GÖGL. Pp. 155; 29 ills. Wien: Wilhelm Maudrich, 1947. (Imported by Grune & Stratton.) Price, $7.75.

Congenital Malformations of the Heart. By HELEN B. TAUSSIG, M.D., Assoc. Prof. of Pediatrics, Johns Hopkins Univ. School of Medicine. Pp. 618; 223 ills., 46 in color. New York: The Commonwealth Fund, 1947. Price, $10.00.

Mental Health. By JOHN H. EWEN, F.R.C.P.E., D.P.M., Physician and Lecturer in Psychological Medicine,, Westminster Hosp.; Med. Superintendent, Springfield Mental Hosp., London. Pp. 270. Balt.: Williams & Wilkins, 1947. Price, $4.00.

MENTAL health problems have become a matter of national concern, and this book offers fundamental knowledge to the student, the general practitioner, those dealing with social and educational problems, child welfare, delinquency and special matters of industry. Preference is shown for the term, psychological medicine, since subjects may range from mild eccentricities to major psychoses. The sections on psychoneuroses and psychoses are concise and lucid. Almost 25 pages are given to the various English legal aspects, which laws the writers hope may be improved. Obviously, the section does not meet our needs; otherwise, the book is admirable. N. Y.

Dermatology for Nurses. By G. H. PERCIVAL, M.D., PH.D., Grant Prof. of Dermatology, Univ. of Edinburgh, and ELIZABETH TODDIE, S.R.N. Pp. 116; 73 ills. Balt.: Williams & Wilkins, 1947. Price, $4.50.

"THE aim of this book is to present a detailed account of the nursing of the common diseases of the skin for nurses who have received no systematic teaching in the subject." (Preface.)

Radium Dosage. The Manchester System. Edited by W. J. MEREDITH, M.Sc., F.INST.P., Christie Hospital and Holt Radium Institute, Manchester. Pp. 124; 4 plates. Balt. and London: Williams & Wilkins, 1947. Price, $4.50.

"THE purely physical aspects of the work can be found in Part II which consists of the physical sections of the papers presented almost completely in their original form." (Preface.)

Psychopathology and Education of the Brain-injured Child. By ALFRED A. STRAUSS and LAURA E. LEHTINEN. Pp. 206; 46 ills. New York: Grune & Stratton, 1947. Price, $5.00.

"THIS book deals with those children, physically handicapped or physically sound, who show intellectual and personality aberrations as a result of injury to the brain substance." (Introduction.)

Manual de Micologia Clinica. By NORMAN F. CONANT, PH.D., DONALD STOVER MARTIN, M.D., DAVID TILLERSON SMITH, M.D., ROGER D. BAKER, M.D., and JASPER LAMAR CALLAWAY, M.D., Traducción y adapteión por el PROF. DR. GUSTAVO PITTALUGA. Pp. 456; 148 ills. Havana, Cuba: M. V. Fresneda, 1947. Price not given.

ANOTHER volume of the series published under the auspices of the Division of Medical Sciences of the National Research Council to disseminate information of value in military medicine.

NEW EDITIONS

Gynecological and Obstetrical Urology. By HOUSTON S. EVERETT, M.D., Assoc. Prof. of Gynecology, the Johns Hopkins Univ. 2nd Ed. Pp. 539; 232 ills. Balt.: Williams & Wilkins, 1947. Price, $6.00.

Public Health Administration in the United States. By WILSON G. SMILLIE, M.D., DR.P.H., Prof. of Public Health and Preventive Medicine, Cornell Univ. Medical College. 3rd Ed. Pp. 637. New York: Macmillan, 1947. Price, $6.50.

Biochemistry for Medical Students. By WILLIAM VEALE THORPE, M.A. (CANTAB.), PH.D. (LOND.), Reader in Chemical Physiology, Univ. of Birmingham. 4th Ed. Pp. 496; 36 ills. Balt. and London: Williams & Wilkins, 1947. Price, $5.00.

"A NEW chapter on the Use of Isotopes in Biochemical Investigations, which could not be included in the previous edition owing to paper restrictions, has now been added. Much other new material has been incorporated and the sections on protein structure, co-enzymes, flavoproteins, bile pigments and nutrition in wartime have been largely rewritten." (Preface.)

Recent Advances in Sex and Reproductive Physiology. By J. M. ROBSON, M.D., D.Sc., F.R.S.E., Reader in Pharmacology, Guy's Hospital Medical School, Univ. of London. 3rd Ed. Pp. 336. Phila.: Blakiston, 1948. Price, $5.75.

Blood Pressure and Its Disorders, Including Angina Pectoris. By JOHN PLESCH, M.D., formerly Prof. of Internal Medicine, Univ. of Berlin. 2nd Ed. Pp. 307; 125 figs. Balt.: Williams & Wilkins, 1947. Price, $6.00.

A Text-Book of Bacteriology. By R. W. FAIRBROTHER, M.D., D.Sc. (MAN.), F.R.C.P. (LOND.), Special Lecturer in Bacteriology, Univ. of Manchester. 5th Ed. Pg. 480; 6 ills. New York: Grune & Stratton, 1948. Price, $6.00.

Treatment of Some Chronic and "Incurable" Diseases. By A. T. TODD, O.B.E., M.B. (EDIN.), M.R.C.P. (LOND.). 2nd Ed. Pp. 324. Balt.: Williams & Wilkins, 1947. Price, $7.00.

Recent Advances in Pathology. By GEOFFREY HADFIELD, M.D., F.R.C.P., Prof. of Pathology, Univ. of London, and LAWRENCE P. GARROD, M.D., F.R.C.P., Prof. of Bacteriology. 5th Ed. Pp. 362; 60 ills. Phila.: Blakiston, 1948. Price, $6.00.

THIS volume differs from previous editions by the addition of a new chapter on the liver, and the inclusion of recent experimental work on cancer and on inflammation. The authors attempt to summarize important developments of the last 2 decades in selected major fields. By and large, the volume contains little that does not appear in recent editions of the standard textbooks. A. R.

A Text-book of Mental Deficiency (Amentia). By A. F. TREDGOLD, M.D., F.R.C.P., F.R.S. (EDIN.), Consulting Physician to Univ. College Hospital, London. 7th Ed. Pp. 534; 47 ills. Balt.: Williams & Wilkins, 1947. Price, $8.50.

THIS is a book of recognized value, and the current edition, which includes much new material, is planned to meet the needs of the psychiatrist and psychologist, the general practitioner, and the medical officers of public authorities and institutions. The English laws, included, are not wholly applicable here. N. Y.

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